PHYSIOLOGICAL EFFECTS OF ENVIRONMENTAL FACTORS AND GROWTH REGULATORS ON FLORAL INITIATION AND DEVELOPMENT OF PINEAPPLE [*ANANAS COMOSUS* (L.) MERR.]

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ABSTRACT

To improve the understanding of environmental control of pineapple flowering and the ability to manipulate this process, the effects of temperature, water stress, and growth regulators on natural flowering or ethephon-induced flowering of 'Smooth Cayenne' pineapple were studied. A night temperature of 30 °C reduced pineapple CO_2 dark fixation relative to that 20 °C. Plants grown at a 30 °C night temperature tended to produce less ethylene and had lower 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACCOase) activity. Plants transferred from a 30/20 to a 30/30 °C day/night temperature regime immediately after treatment with ethephon were not induced to flower. Plants grown in the constant 30/30 °C regime were forced but produced fruits with significantly fewer fruitlets per fruit than did plants grown in a 30/20 °C environment. Both water excess and water deficit stress significantly reduced dark CO_2 fixation. Water excess stress imposed by flooding pots three times per day promoted ethylene production and ACCOase activity of leaf basal-white (basal) tissue, but has no effect on ethylene production and ACCOase activity of stem apical (stem) tissue. Water deficit stress significantly decreased ethylene production by leaf basal tissue and ACCOase activity of leaf basal and stem tissues, but had no effect on ethylene production by stem tissue. Neither water excess nor water deficit stimulated natural flowering. However, both water excess and water deficit imposed after forcing with ethephon reduced fruitlet number and fruit size relative to the control. Fruitone, paclobutrazol and uniconazole delayed or inhibited flowering, while aminooxyacetic acid, aminoethoxyvinylglycine, daminozide, and silver

thiosulfate had no effect. Uniconazole and paclobutrazol inhibited ethylene production and ACCOase activity of leaf basal tissue, which may account for their effect on flowering. Fruitone stimulated ethylene production and increased ACCOase activity and malonyl-ACC (MACC) content in stem tissue, but the mechanism by which it delayed or inhibited flowering is not known. It is concluded that a 30 °C night temperature decreased CO_2 fixation and reduced the flower induction response and fruitlet numbers. Water stress clearly is not a main factor in controlling flower induction. Fruitone, uniconazole and paclobutrazol delay or inhibit flowering of pineapple.

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LIST OF ABBREVIATIONS

ACC, 1-Aminocyclopropane-1-carboxylic acid

AOA, Aminooxyacetic acid

AVG, Aminoethoxyvinylglycine

Daminozide, Butanedioic acid mono-(2,2-dimethylhydrazide)

Ethephon, (2-Chloroethyl)phosphoric acid

Fruitone, 2-(3-Chlorophenoxy) propanoic acid

MACC, N-malonyl-(1-aminocyclopropane-1-carboxylic acid)

NAA, α-Naphthaleneacetic acid

Paclobutrazol, (2RS,3RS)-1-(4-chlorophenyl)methyl]-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol

RWC, Relative water content

STS, Silver thiosulfate

TA, Titratable acidity

Uniconazole, (E)-(p-Chlorophenyl)-4,4-dimethyl-2(1,2,4-triazol-1-yl)-penten-3-ol

CHAPTER 1

INTRODUCTION

Pineapple (*Ananas comosus* [L.] Merr.) is an economically important fruit crop cultivated in tropical and subtropical regions. On plantations and farms, growth regulators such as the salt of naphthaleneacetic acid (NAA), ethylene, ethephon, and calcium carbide are used to synchronize flower induction (forcing) (Traub et al., 1939; Clark and Kerns, 1942; Bartholomew and Criley, 1983). This practice makes pineapple management much easier because fruits can be produced throughout the year and harvest dates can be scheduled.

Since forcing has become a common commercial practice in pineapple cultivation and management, flowering that occurs before the scheduled forcing date is not desirable. In some areas and in some years, precocious flowering may cause serious yield losses because it produces fruits that are either too small or too labor-consuming to harvest. Also, sporadic flowering interferes with the prediction of fruiting and yield.

Pineapple plants that can be induced to flower with growth regulators are said to be susceptible to forcing. In areas having a high night temperature, plants sometimes are not easily forced by growth regulators (Bartholomew and Malézieux, 1994). The internal factors controlling plant susceptibility to forcing are still unknown. The biochemical mechanisms controlling the transition of a meristem from

a vegetative to a reproductive phase in higher plants are also unknown and several hypotheses have been proposed (Bernier, 1988). They include the florigen/anti-florigen concept, electrical signals, nutrient diversion hypothesis, and the multifactorial model (Bernier, 1988).

Although pineapple is characterized as a quantitative short-day plant (Gowing, 1961; Friend and Lydon, 1979), and low night temperature promotes natural flowering (Van Overbeek and Cruzado, 1948a) and increases the chance of a successful forcing with growth regulators (Bartholomew and Malézieux, 1994), the mechanism controlling the flowering of this plant needs to be further elucidated. Understanding the physiological mechanism(s) controlling flowering and plant susceptibility may improve the success in manipulating flower induction and fruit development in pineapple.

Ethylene is a plant hormone that regulates plant growth and development. The biosynthesis and regulation of ethylene has been widely studied, and water deficit stress, waterlogging, temperature, heavy metal ions, gamma radiation, and mechanical stress all influence its production (Abeles et al., 1992; Mattoo and Suttle, 1991). Environmental factors that influence plant ethylene production may affect natural flowering of pineapple. The effects of temperature and water stress on CO_2 dark fixation as evidenced by leaf titratable acidity, an easily measurable indicator of the intensity of Crassulacean acid metabolism (CAM) (Winter, 1985), ethylene metabolism, and flowering and fruit development need to be investigated. The research was designed to test the following hypotheses:

1. High night temperature decreases plant susceptibility to forcing due to reduced tissue ethylene production and CO_2 assimilation, as indicated by leaf titratable acidity (TA) measured at the end of dark period (am TA).

2. Water stress (deficit and excess) promotes pineapple flowering by increasing tissue ethylene production.

3. High night temperature and water stress reduce fruitlet number and fruit size due to decrease in CO_2 dark fixation.

4. Growth regulators that inhibit ethylene production or block ethylene action will inhibit environmentally induced (natural) flowering of pineapple.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Flowering Induction in Pineapple

Pineapple is the second most important crop economically in Hawaii. Regulation of pineapple flowering is important because natural flowering in Hawaii, as well as in other pineapple growing regions, is sporadic (Bartholomew and Kadzimin, 1977). The technology of artificial induction of flowering, also commonly called forcing, with chemicals such as ethylene, ethephon, NAA, and calcium carbide is well developed (Bartholomew and Criley, 1983). Nevertheless, plants growing rapidly or being grown in an environment with a high night temperature are more difficult to force in the field. Another problem in pineapple producing areas is that small plants sometimes flower naturally, producing unmarketable fruits. The physiology of pineapple flowering and the role of auxin and ethylene will be briefly reviewed.

2.1.1 Flowering of pineapple in response to environmental factors

Prior to the emergence of the young inflorescence in the plant heart, which occurs about 2 months after floral initiation (Bartholomew, 1977), it is difficult to distinguish between a vegetative and a reproductive pineapple plant without sacrificing it. Pineapple tends to flower in the late fall to early winter but flowering can occur at any time of the year. The duration from planting to natural floral initiation is

dependent on the weight or size of the material planted (Collins, 1960; Py et al., 1987) as well as climatic changes (Bartholomew and Kadzimin, 1977).

Generally plant size or age determines to some extent the susceptibility or "ripeness to flower" of 'Smooth Cayenne' to environmental floral signals (Bartholomew and Kadzimin, 1977). With daylength being an important factor in floral initiation, Gowing (1961) and Friend and Lydon (1979) classified 'Smooth Cayenne' pineapple as a quantitative but not an obligate short-day plant. Under an 8-hour daylength flowering was most rapid at a night temperature of 20 °C compared with plants grown at 15 and 25 °C; flowering did not occur after 3 years of growth at 30 °C (Friend, 1981). 'Red Spanish' pineapple did not respond to reduced day length but flowering was promoted if plants were exposed to lower night temperature (Van Overbeek and Cruzado, 1948a). Williams (1987) stated that floral differentiation of some varieties in his breeding program in Hawaii were totally dependent on size, observing that each plant differentiated when it reached about 1.5 kg in weight, regardless of the changes in day length and day-night temperature; however, no detailed data were given.

Plants growing rapidly tend to be less sensitive to environmental floral stimulation. A large amount of nitrogen fertilizer and optimum water supply promote vegetative growth and thus inhibit flowering (Bartholomew and Kadzimin, 1977; Bartholomew and Malézieux, 1994). Factors that retard vegetative growth, i.e., a decrease in nitrogen or water supply and reduced temperature, day length, and solar radiation promote flowering (Bartholomew and Kadzimin, 1977). It was speculated

that in some cases flower induction was related to plant growth retardation due to root damage caused by pests and disease (Williams, 1987).

It was recently hypothesized that natural floral initiation occurs in response to naturally produced ethylene, or to an increase in plant susceptibility to that ethylene, or both (Bartholomew, unpublished data, 1991; Min and Bartholomew, 1993; see Appendix). Williams (1987) speculated that stress-produced ethylene initiated some precocious flowering.

2.1.2 Induction of flowering in pineapple with growth regulators

The history and development of flowering induction in pineapple with growth regulators was comprehensively reviewed recently (Bartholomew and Kadzimin 1977; Bartholomew and Criley, 1983; Williams, 1987). Growth regulators, such as naphthaleneacetic acid (NAA, or its sodium salt, SNA), β-hydroxyethylhydrazine (BOH), 2,4-dichlorophenoxyacetic acid (2,4-D), ethephon [(2-chloroethyl) phosphonic acid], ethylene, indoleacetic acid (IAA), naphthaleneacetamide, acetylene and calcium carbide could force flower development of pineapple (Gowing and Leeper, 1960; Bartholomew and Criley, 1983). However only calcium carbide, ethephon, ethylene, and NAA (or SNA), have been used in commercial practice to force pineapple plants (Bartholomew and Criley 1983).

As noted previously, the susceptibility of pineapple to forcing initially depends on the age or size of the plants. Conway (1977) found that 2 month-old plants (about 500 g fresh weight) grown from crowns could not be forced with ethephon regardless of the night temperature, while 4-month old plants (about 800 to 900 g fresh weight)

were readily forced at a night temperature of 20 ° C, but were less or not susceptible at night temperature of 25 or 30 °C. In commercial practice, in order to produce marketable fruits, plants need to reach a minimum weight before forcing, because the weight of the plant and the fruit are highly correlated (Dass et al., 1977; Bartholomew and Criley, 1983; Py et al., 1987; Bartholomew and Kadzimin, 1977). Usually larger plants are easier to force than small ones, but very large plants are also reported to be difficult to force (Bartholomew and Criley, 1983). It seems that plant susceptibility to forcing is related to physiological status of the plant and not just size or age.

Recently the relationships between plant size (700 to 1300 g), ethylene production and 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACCOase) activity in D-leaf (the longest leaf on the plant) basal and stem tip tissues was examined using pot-grown plants (Min and Bartholomew, 1993; see Appendix). Ethylene production in D-leaf basal tissue was well correlated with plant size, but ethylene production in stem tip tissue and ACCOase activity in both tissues was not. Ethylene production and ACCOase activity was well correlated in D-leaf basal tissue but not in stem apical tissue. Little ethylene was produced by green leaf tissue.

De Greef et al. (1983) reported that flowering maturity of *Aechmea victoriana*, an ornamental bromeliad, was correlated with the plant's capacity to convert applied ACC to ethylene. However no relationship between ethylene production and plant size was found.

Pineapple plants are more susceptible to forcing near the season of natural

floral initiation and this is especially evident when NAA is used (Bartholomew and Kadzimin, 1977). Cooper (1942) found that pineapple plants in Florida were induced to flower in the fall with NAA while summer induction failed. Ethylene, on the other hand, induced flowering equally well in the summer and fall (Cooper, 1942). Wee and Rao (1977) reported that short day length and low maximum temperature improved the flowering response of plants to growth regulators as well as stimulated natural flower initiation. A night temperature of 25°C or greater reduced the susceptibility of 'Smooth Cayenne' to forcing with ethephon (Conway, 1977; Bartholomew and Kadzimin, 1977). Plants with a high level of nitrogen in the leaf tissue also tend to have reduced susceptibility to forcing. Therefore, in the field, stopping nitrogen fertilizer and irrigation about one month prior to forcing can increase the percentage of initiation in areas where forcing is expected to be difficult (Bartholomew and Kadzimin, 1977).

The mechanism(s) of pineapple flower induction with growth regulators, which involves a change from the vegetative to the reproductive (floral initiation) stage in pineapple plants has not yet been characterized. Gowing (1956) hypothesized that exogenously applied auxin competitively lowered the effective endogenous auxin level, but this hypothesis has never been confirmed. Burg and Burg (1966) showed that auxin stimulated ethylene production, which was assumed to cause pineapple to flower. Recent work showed that ethylene production by stem tip tissue from NAA-treated plants was much higher than that produced by stem tip tissue of the control (Min and Bartholomew, 1993; see Appendix).

Green leaf tissue is required for flower initiation with gaseous ethylene (Traub et al., 1939). When all the mature leaves of plants were removed, leaving only the short basal parts of mature leaves and the etiolated young leaves in the heart of the plant, no floral initiation occurred as a result of applying ethylene. Plants defoliated to one large leaf before treatment, and plants totally defoliated one or two days after treatment, initiated flower buds (Traub et al., 1939).

Cooper (1939) studied the normal distribution of auxin in the pineapple plant and showed that this substance was concentrated in young etiolated leaves and the upper part of the stem. Ethylene did not influence the distribution of auxin (Traub et al, 1939). However, the flowering of the horizontally placed 'Cabezona' pineapple indicated the possible involvement of auxin in flowering control because auxin redistribution occurred as a result of geotropic stimulation (Van Overbeek and Cruzado, 1948b).

Pineapple is a plant where the apical meristem changes from vegetative to reproductive development, after which it reverts to vegetative growth. The early development of the inflorescence of pineapple forced with growth regulators was observed by Chin (1975) and Bartholomew (1977). About 6 to 8 days after application of forcing agents the first bract primordium could be seen; a complete flower with sepals and petals could be observed by 20 to 22 days after treatment; and approximately 30 to 45 days after treatment, differentiation of floral primordia stopped (Bartholomew, 1977). The duration of floral differentiation may depend on plant clones (cultivars), plant physiological state, environmental conditions, and the

growth regulator used in forcing. The mechanisms controlling the start and end of floral initiation and reversion to the vegetative state are still unknown.

Attempts to chemically delay flowering have met with limited success. A high concentration and repeated application of NAA inhibited flowering (Gowing, 1956; Leeper, 1965; Millar-Watt, 1981). But NAA was never used commercially to delay flowering because of the possibility of drift and subsequent forcing of adjacent fields (Leeper, 1965). Scott (1992) found that spraying 3000 L/ha of a solution containing 50 ppm (active ingredient, a.i.) of 2-(3-chlorophenoxy)propionic acid (Fruitone, Fruitone CPA) decreased precocious flowering from 48.5% to 8.2% in a ratoon field. Millar-Watt (1981) reported that only about 27% of plants flowered if they were sprayed 3 times at monthly intervals with 1000 ppm silver nitrate solution while 57% of control plants flowered. Silver ion is a powerful inhibitor of ethylene action (Beyer, 1976). Sanford and Bartholomew (1981) found that silver nitrate applied a few hours prior to ethephon forcing inhibited floral induction by ethephon. Recent work confirmed the inhibitory effect of silver ion on ethephon forcing (Min and Bartholomew, 1993; see Appendix). Silver thiosulfate (20 mg/plant in 10 ml water, a mixture of silver nitrate and sodium thiosulfate in a 1:4 molar ratio) applied 7 days prior to ethephon application inhibited floral induction by ethephon. It may be useful to further evaluate the potential of silver thiosulfate to inhibit natural flowering.

2.1.3 Biosynthesis of ethylene and its role in pineapple flowering

The biological effects, biosynthesis, and regulation of ethylene have been fully reviewed (Yang and Hoffman, 1984; Shimokwa, 1984; Morgan, 1976; Yang,

1987; Veen, 1987; Mattoo and Suttle, 1991; Abeles et al., 1992). The pathway of ethylene biosynthesis is generally accepted to be as follows:

Methionine $\rightarrow^{(1)}$ S-adenosylmethionine (AdoMet, SAM) $\rightarrow^{(2)}$ 1-aminocyclopropane-1-carboxylic acid (ACC) $\rightarrow^{(3)}$ ethylene.

There are three enzymes, AdoMet synthetase[(1)], ACC synthase[(2)] and ACCOase (ethylene-forming enzyme, EFE)[(3)] involved in the biosynthetic process (Kende, 1989; Kende, 1993). ACC synthase was found to be induced by those factors that promote ethylene formation, e.g. by auxin and by stress, such as wounding (Yang and Hoffman, 1984). The ACC level can be regulated by conversion of ACC to malonyl-ACC (MACC), a stable product that can not be converted to ACC. The reaction of ACC conjugation is catalyzed by ACC malonyltransferase (Hoffman et al., 1983; Yang et al., 1990).

Many compounds have been shown to inhibit ethylene biosynthesis or block its action. These compounds include aminoetheoxyvinylglycine (AVG), aminooxyacetic acid (AOA), cobalt ion, free radical scavengers such as propyl gallate, low O_2 atmospheres, polyamines, and uncouplers such as 2,4-dinitrophenol (DNP) (Wang, 1987). Plant growth retardants such as uniconazole also inhibited ethylene production (Grossmann et al., 1989; Hofstra et al., 1989; Kraus et al., 1991). Silver ion (silver nitrate and silver thiosulfate) (Beyer, 1976; Veen, 1987; Knee, 1992), 2,5-norbornadiene and related cycloolefines (Sisler and Wood, 1988) are ethylene action inhibitors. The mechanism of ethylene action at the molecular level in the plant has been extensively studied (Shimokwa, 1984; Yang, 1987; Abeles et al., 1992) but still

is not elucidated. The biological activity of ethylene is thought to result from its binding to a receptor, where it forms an activated complex, which in turn initiates the reactions leading to a wide variety of biological responses (Yang, 1987). Recent success in cloning the genes responsible for ethylene sensitivity from mutants may help to identify the ethylene signal pathway (Jones, 1994).

There is no doubt that exogenously applied ethylene regulates floral initiation of pineapple (Bartholomew and Criley, 1983), as well as other bromeliads (Meker et al., 1983; De Greef et al., 1989). Meker et al. (1983) found 10 ppm AVG was active in preventing flowering of ornamental Bromeliaceae, and there were no adverse effects on inflorescence development following ethephon spray two weeks after AVG treatment. All evidence shows that ethylene is involved in the floral initiation of pineapple and ornamental Bromeliaceae.

2.2 Overview of Physiological Mechanism(s) of Floral Initiation in Higher Plants

Flowering of higher plants is an extremely complicated process. No attempt was made here to review all the literature concerning flowering physiology because this topic has been well and comprehensively reviewed (Bernier et al., 1981a; Bernier et al., 1981b; Kinet et al., 1981; Bernier, 1988; Schwabe, 1987; Bernier et al., 1993; Kinet, 1993; Thomas, 1993). The main points and recent advances in this area will be covered here.

The process of flowering is generally divided into two major phases, flower initiation and flower development. Evocation, defined as the processes in the apex

required for irreversible commitment to initiate flower primordia, is the first step for the initiation of flowers (Kinet et al., 1981). Environmental factors responsible for flowering induction mainly are daylength - photoperiodic responses (photoperiodism) and temperature - chilling effects (vernalization) (Wareing and Phillips, 1981; Bernier, 1988). Generally, once a plant is sufficiently mature to be induced to flower, a leaf is needed for the perception of photoperiodic stimuli and the shoot apical meristem is most sensitive to vernalization (Wareing and Phillips, 1981; Bernier, 1988). Based on the results of grafting studies in photoperiodically sensitive plants, Chailachjan (1936) postulated the presence of a "florigen", a hypothetical flowering hormone, that was synthesized in the leaves under favorable daylength conditions and transmitted to the growing point. Based on the effects of chilling on flower induction, the concept of "vernalin" was postulated and the relationship between vernalin, florigen and flower formation was discussed (Lang, 1965). Neither of these hypothetical agents has ever been identified (Bernier, 1988).

Theories of internal control of evocation were fully reviewed (Zeevaart, 1976; Benier, 1988). These theories include the florigen/antiflorigen concept, electrical signals, nutrient diversion hypothesis, and the multifactorial control model (Bernier, 1988). Among these hypotheses, florigen/antiflorigen and the multifactorial control model dominate, but still need to be proved. The multifactorial control model proposed by Bernier et al. (1981b) is summarized as follows:

Evocation is controlled by several factors that are either positive or negative. Whether the evocational processes are activated or not depends on the balance of

promoters and inhibitors. These factors are not necessarily the same in different species, and they may be synthesized in the leaves, the roots, the apex, or elsewhere. When one or several factors are absent, the process of evocation can not proceed. The number and nature of the missing or limiting factors in unfavorable conditions depends on the species and environment. All required factors exist in inductive conditions.

O'Neill (1992) summarized the recent advances in the photoperiodic control of flowering and concluded that "any explanation of the mechanism of photoperiodic flowering must account for the presence of both promoters and inhibitors in photoperiodic plants." However, Bernier et al. (1993) recently presented results to further support the theory of multifactorial control of flowering.

As noted previously, floral initiation of pineapple is controlled by internal factors such as age or size of the plants, and external factors such as daylength and temperature. When very small plants were forced with ethephon, bract-like leaves were produced and the shoot apex was elongated (Conway, 1977). This result indicates that partial evocation of the stem meristem occurs in response to ethephon treatment. Vegetative inflorescences in subminimally-induced plants of Kalanchoe, Bryophyllum, and Dianthus were also reported (Bernier, 1992). This phenomena means that the flowering process at the meristem may consist of several independent steps that can be activated individually (Bernier, 1992). Many questions need to be answered such as what these factors are and how evocation could occur. Recently more attention has been paid to plant reproduction including flowering (Chasan and

Walbot, 1993; O'Neill, 1992; 1993), and the hope is that it will lead to an understanding of these processes.

CHAPTER 3

EFFECTS OF TEMPERATURE ON ETHYLENE PRODUCTION, LEAF TITRATABLE ACIDITY, FLORAL INITIATION AND DEVELOPMENT

3.1 Introduction

The mechanism(s) controlling the transition of the plant apical meristem from a vegetative to a reproductive state, generally termed floral initiation or evocation, is still unknown (Bernier, 1988), but day length and temperature are the controlling environmental factors. 'Smooth Cayenne', the most widely cultivated pineapple group, has been classified as a quantitative short-day plant (Gowing, 1961; Friend and Lydon, 1979). Plants grown in 8-hour days flowered earlier than did plants grown in longer days (Friend and Lydon, 1979). Plants grown at 20 °C flowered earlier than did those grown at 15 or 25 °C and plants did not flower after 3 years growth at 30 °C (Friend, 1981). With plants of the 'Red Spanish' group, 88% flowered after a one-month exposure to a 16.7 °C night temperature while only 28% of the plants exposed to 22.2 °C flowered (Van Overbeek and Cruzado, 1948a).

The growth regulators ethylene, naphthaleneacetic acid (NAA), and ethephon are used commercially to force flowering (Bartholomew and Criley, 1983). Plants grown at warm night temperatures (above about 24 °C) are more difficult to force than are plants grown at cooler temperatures (Bartholomew and Malézieux, 1994). Failure of the forcing treatment commonly occurs in Australia during the hot summer

season (Turnbull et al., 1993) and in locations having high night temperatures unless special precautions are taken (Bartholomew and Malézieux, 1994). Even where forcing is successful, average fruit weight may be reduced (Bartholomew and Malézieux, 1994).

In order to better understand and manipulate flowering, the effects of high night temperature on flowering and the production of ethylene, which is assumed to be the stimulus for natural flowering of pineapple (Min and Bartholomew, 1993; see Appendix), need to be studied further. The objectives of this study were to characterize the effects of high night temperature on tissue ethylene production, 1-aminocyclopropane-carboxylic acid (ACC) oxidase (ACCOase), also called ethylene forming enzyme (EFE), activity, plant susceptibility to forcing with ethephon, and subsequent inflorescence development.

3.2 Materials and Methods

Plant materials and treatments

'Smooth Cayenne' pineapple clone Champaka F-153 was used in all experiments.

Experiment 3.1 Suckers weighing approximately 225 g were planted on June 16, 1992 in 8.5 L (23.2 cm diameter by 20 cm high) pots filled with a 1:1 (by volume) mixture of Sunshine #4 (a commercial mixture) and horticultural perlite. The plants were grown in a glass-house. Each plant was fertilized once per two weeks with 1.0 g Gaviota Foliar 62 (Brewer Environmental Industries, Honolulu), a

commercial soluble fertilizer (N 12%, P_2O_5 24%, K_2O 24%, Mg 0.04%, Fe 0.1%, Cu 0.013%, B 0.01%, Mo 0.02%, Mn 0.012%, Zn 0.142%), 0.04 g $CaCl_2$ and 0.3 g urea dissolved in 250 ml water beginning one month after planting. The plants were watered about once every 10 days.

On January 4, 1993 (202 days after planting), 16 plants were placed in each of two growth chambers maintained at day/night temperatures of 30/20 or 30/30 °C. The photoperiod was 12 hours and the photosynthetic photon flux at mid-plant height was 520 μmol m^{-2} s^{-1}. Humidity was not controlled. Thirty days later, 4 plants from each environment (30/20 or 30/30) were removed for measurement of ethylene and ACCOase activity of the apical 1 cm of the stem (stem tissue) and the white basal tissue (basal tissue) of the D-leaf (the longest leaf on the plant) or the leaf just younger than the D-leaf (D+1).

The remaining 12 plants in each environment were treated by pouring 10 ml of a 2% urea-water solution containing 10 mg of ethephon into the center of each plant. Six plants from each environment were then moved to the opposite temperature regime resulting in treatments consisting of: 1) continuous growth at 30/20 °C (30/20), 2) continuous growth at 30/30 °C (30/30), 3) growth at 30/20 °C up to the time of forcing and growth at 30/30 °C thereafter (30/20-30/30) and 4) growth at 30/30 °C up to the time of forcing and growth at 30/20 °C thereafter (30/30-30/20). Sixty days after forcing, percentage of plants induced to flower, fruitlet number per inflorescence, and inflorescence dry weights were recorded.

Experiment 3.2 With minor exceptions, experiment 3.2 was a repeat of the

first experiment. Crowns weighing approximately 168 g were planted on September 12, 1992. The plants were moved into growth chambers 203 days after planting. The conditions and numbers of plants were the same as for Exp. 3.1, but the photosynthetic photon flux was 470 μmol m^{-2} s^{-1}. Four plants from each treatment were sampled for ethylene production and ACCOase activity of the stem and basal tissue at 31 and 51 days after treatment. The 8 remaining plants were forced as in Experiment 3.1 and half (4) of the plants were placed in the opposite temperature regime. The plants were harvested 66 days after forcing. The plant fresh weight (roots not included), inflorescence fresh and dry weight, fruitlet number, stem fresh and dry weight were recorded.

Experiment 3.3 Crowns weighing approximately 125 g each were planted on April 25, 1993 and maintained as in experiment 3.1. Seven plants were moved on April 18, 1994 into each of two controlled environments maintained at 30/20 or 30/30 °C day/night temperatures. A 12-h photoperiod was used and the photosynthetic photon flux was 470 μmol m^{-2} s^{-1}. The plants were forced by pouring 15 ml of a 2% urea water solution containing 15 mg ethephon into the plant center the day the plants were moved into the growth chambers. Leaf relative water content (RWC) and titratable acidity (TA) were measured 21 and 38 days, respectively, after ethephon treatment. Plants were harvested 81 days after forcing and data were taken on plant fresh and dry weight (roots not included), leaf area, and fruitlet number.

Measurement of ethylene and ACCOase activity

In an evaluation of ethylene production rates by different pineapple tissues,

leaf basal white tissue was found to produce more ethylene than leaf white-green or green tissues, and the ethylene produced by the D-leaf was greater than that produced by younger or older leaves (Min and Bartholomew, 1993; see Appendix). Therefore, in this study the D- and D+1 leaves were used for the measurement of ethylene and ACCOase. Four plants from each treatment were sampled. Approximately 2 cm of leaf basal white tissue was removed and divided longitudinally into two parts; one part was used for ethylene measurement and the other for the measurement of ACCOase activity. The upper 1.0 cm of stem apical tissue was divided into two parts for ethylene and ACCOase measurement. About 1.0 g of tissue was put into a 17.5 ml test tube, sealed with a serum stopper and incubated for about 2 hours (exceptions will be noted) at room temperature (about 25 °C) and in room light (about 10 μM $m^{-2}s^{-1}$). After incubation, a 1.0 ml gas sample was withdrawn from the headspace and injected into a gas chromatograph (GC) equipped with a flame ionization detector (Min and Bartholomew, 1993; see Appendix). Although a wounding effect was not avoided, it was minimized by incubating tissues for about 2 hours. The pre-study showed that ethylene production rates of different pineapple tissues increased in parallel for up to 5 hours of incubation.

ACCOase, which converts ACC to ethylene, does not survive homogenization because it is membrane-bound and its activity requires membrane integrity (Yang and Hoffman, 1984; Kende, 1989). The properties of this enzyme have been elucidated *in vivo* and in vacuolar preparations that possess the capacity to convert ACC to ethylene (Kende, 1989). The *in vivo* ACCOase activity (ACCOase activity) was estimated by

measuring the conversion of ACC to ethylene in intact tissue as has been done by others (Larriguadiere et al., 1991; Gallardo, et al., 1994; Starrett and Laties, 1990). The procedure used in this study was modified from that of Starrett and Laties (1991). About 1.0 g of fresh tissue was dipped in a 1.0 mM ACC solution for 2 minutes, blotted dry, placed in a 17.5 ml test tube, sealed with a serum stopper, and incubated for approximately 2 hours as described for the measurement of ethylene production, after which ethylene was measured as described above.

Measurement of leaf relative water content (RWC) and titratable acidity (TA)

Leaf discs (about 2.0 cm^2) were sampled from the middle part of the D-leaf. RWC was measured following the procedure of Barrs and Weatherley (1962) and George et al. (1984). After obtaining the fresh weight (FW), one leaf disc from each plant was floated on deionized water in a petri dish for 4 hours at room temperature (25 °C) and light (10 μM $m^{-2}s^{-1}$). The discs were blotted dry and re-weighed to obtain the turgid weight (TW), then oven dried for 24 hours at 70 °C. After measuring the dry weight (DW), RWC was calculated by the formula:

RWC(%)=(FW-DW)/(TW-DW)*100.

To measure TA, leaves were sampled at the end of the dark period (am) and at the end of light period (pm). Two leaf discs from the middle one-third of the D-leaf were ground in a mortar and brought to about 50 ml with deionized water, and boiled for 10 minutes. After cooling, the sample was titrated with 0.01 N NaOH to pH 8.0 (Modified from Friend and Lydon, 1979).

3.3 Results

3.3.1 Ethylene Production and ACCOase activity

In Experiment 3.1, ethylene production and ACCOase activity of leaf basal-white and stem apical tissue of plants grown in the 30/30 treatment were lower than in the same tissues of plants grown in the 30/20 treatment (Table 3.1) and most differences were significant. In Experiment 3.2, after 31 and 51 days growth, tissue ethylene production and ACCOase activity in tissue from plants grown in the 30/30 and 30/20 treatments were not significantly different, but values in the 30/20 treatment were consistently higher than those for plants in the 30/30 treatment (Table 3.1).

3.3.2 Leaf RWC and TA

Plants in the 30/30 treatment had significantly lower leaf RWC and am TA ($P = 0.01$), but pm TA values were not different (Exp. 3.3) (Table 3.2). The difference between the am and pm TA (net TA) values, which indicates the capacity for CO_2 dark fixation of plants, was significantly reduced in the 30/30 treatment (Table 3.2).

3.3.3 Fruit initiation, fruitlet number and fruit growth

After plants in Experiment 3.1 were placed in the growth chambers and treated with ethephon, some initiated flower development naturally and these were excluded from the analysis (Table 3.3). These plants were identified by emergence of an inflorescence 14 to 21 days after ethephon treatment rather than 55 or more days (Bartholomew, 1977). This flowering was assumed to be a response to the cool night temperatures and short daylengths prevailing in the winter in Hawaii. All plants but

one in the 30/20-30/30 treatment were forced with ethephon. Two plants in the 30/30 treatment in this experiment produced an inflorescence-like structure with bracts, which normally subtend each fruitlet, but all other flower structures were missing. No natural flowering occurred in Experiment 3.2. In the 30/20-30/30 treatment, the one remaining vegetative plant in Experiment 3.1 was not forced and three of four plants were not forced in Experiment 3.2 (Table 3.3).

Fruitlet number of plants in the 30/20 treatment was significantly greater than that of plants in the 30/30 treatment in both experiments (Table 3.3). Plants in the 30/30-30/20 treatment had fewer fruitlets than did plants in the 30/20 treatment and the difference was significant in Experiment 3.2. The one plant in the 30/20-30/30 treatment that was forced also had a reduced number of fruitlets (Table 3.3). In Experiment 3.3, plants in the 30/20 treatment had significantly more fruitlets than did plants in the 30/30 treatment (Table 3.4).

In Experiment 3.2 and 3.3, plants in the 30/20 treatment accumulated significantly more dry matter in fruits and had a significantly smaller crown than did plants in the 30/30 treatment (Tables 3.3 and 3.4). However, there were no significant treatment effects on fresh or dry weights of whole inflorescence (fruit plus crown) in all three experiments (Tables 3.3 and 3.4).

Plants in the 30/20 treatment in Experiment 3.2 had greater fresh or dry weights of most components than did plants in the 30/30 treatment (Table 3.3) while plants in the 30/30-30/20 treatment were intermediate in weight of the measured components. The differences could have been due to the effects of temperature prior

to forcing. The peduncle fresh and dry weight, stem dry weight, and total plant dry weight (roots not included) in the 30/20 treatment in Experiment 3.3 were greater than for plants in the 30/30 treatment (Table 3.4). There were no other significant differences in plant components. The ratio of fruitlet number/leaf fresh weight or leaf area is a measure of the efficiency of a plant in initiating a fruit. Plants in the 30/20 treatment had the highest number of fruitlets per unit of leaf mass while plants in the 30/30 treatment had the lowest number (Tables 3.3 and 3.4). This result suggests that the reduced fruitlet number in the 30/30 treatment was likely due to reduced leaf CO_2 assimilation rather than to reduced leaf mass or area, a result that is consistent with the effects of temperature on TA (Table 3.2).

Table 3.1. Effects of day/night temperature on ethylene production and ACC oxidase activity of pineapple plant tissues. Plants were grown for 30 days at the treatment temperature prior to sampling in Experiment 3.1. In Experiment 3.2, stem and leaf tissue samples were collected 31 and 51 days after temperature treatments were begun.

Tissue†	Night temperature (°C) 30/20	30/30
	- - - - - - - - nl $gFW^{-1}h^{-1}$ - - - - - - - -	
Experiment 3.1		
D+1-ethylene	3.27‡	0.49**
D-ethylene	2.07	0.38*
S-ethylene	0.65	0.31*
D-ACCOase	21.00	3.01
S-ACCOase	9.93	6.79*
Experiment 3.2 (1st sampling)		
D-ethylene	0.49	0.45
S-ethylene	0.21	0.18
D-ACCOase	9.85	6.59
S-ACCOase	6.05	4.82
Experiment 3.2 (2nd sampling)		
D-ethylene	0.41	0.27
S-ethylene	0.19	0.14
D-ACCOase	9.95	7.15
S-ACCOase	5.29	3.53

† D, D-leaf basal white tissue; D+1, the first younger leaf than D-leaf; S, stem apical tissue. Incubation time was 3 hours for Experiment 3.1 and 2 hours for Experiment 3.2.

‡ Values are means of data from 4 plants.

*, ** t-test significant at the 0.05 or 0.01 level of probability, respectively.

Table 3.2. Effects of day/night temperature on leaf relative water content (RWC) and titratable acidity (TA) of pineapple.

	Temperature (°C)	
	30/20	30/30
RWC (%)	94.5†	91.3**
am TA‡	467.7	283.5**
pm TA	72.5	73.0
Net TA	395.2	210.5**

† Values are means of samples collected 21 and 38 days after initiating the temperature treatments (n=6).

‡ Leaf TA (mM H^+ m^{-2} leaf area) of samples at the end of dark period (am) and at the end (pm) of the light period, net TA is the difference between am and pm measurements.

** Values are significantly different by the t-test at the 0.01 level of probability.

Table 3.3. Effects of day/night temperature on fruit initiation and dry matter accumulation of pineapple. Plants in Experiment 3.1 were forced 30 days after initiating the temperature treatments and harvested 60 days after forcing. Plants in Experiment 3.2 were grown for 31 days at the pre-forcing and 66 days at the post-forcing temperature.

	Temperature (°C)				
Pre-forcing	30/20	30/30	30/30	30/20	LSD
Post-forcing	30/20	30/20	30/30	30/30	(0.05)
	Experiment 3.1				
Total plants	6	6	6	6	
No. flowering naturally†	1	0	1	5	
Forced plants	5	6	5	0	
Fruitlet number	97.6	89.3	13.7		25.0
	Experiment 3.2				
Forced plants (out of 4)	4	4	4	1	
Fruitlet number (FN)	113.3	79.0	37.0	49.0	5.0
Fruit FW (g)	122.5	118.2	76.4	70.9	29.8
Fruit DW (g)	13.2	12.5	6.9	6.4	3.3
Crown FW (g)	4.9	8.6	83.8	70.9	18.5
Crown DW (g)	0.8	1.2	7.4	2.4	2.4
Fruit+crown FW (g)	127.4	126.8	157.4	141.8	ns
Fruit+crown DW (g)	13.9	13.6	14.2	8.8	ns
Peduncle FW (g)	134.1	130.0	58.7	63.0	35.9
Peduncle DW (g)	11.9	11.2	4.1	4.4	3.6
Stem FW (g)	199.6	169.0	113.9	163.0	31.5
Stem DW (g)	33.0	27.7	12.6	20.3	7.4
Leaf FW (g)	1697.3	1660.8	1284.9	1505.5	285.4
Total plant FW (g)	2158.3	2086.6	1617.6	1873.3	343.3
Stem DW/Stem FW (%)	16.4	16.3	11.1	12.4	0.72
FN/Leaf FW (g)	0.066	0.047	0.028	0.032	0.007

† Flower initiation began before ethephon was applied. FW, fresh weight; DW, dry weight.
ns, F-test not significant at the 0.05 level of probability.

Table 3.4. Effects of day/night temperature treatments imposed after forcing on fruit initiation and dry matter accumulation of pineapple. Plants were grown for 81 days at the treatment temperature prior to sampling.

	Night temperature (°C)	
	30/20	30/30
Fruitlet number	101.5†	73.0**
Fruit FW‡ (g)	392.0	269.6**
Crown FW (g)	60.2	214.1**
Fruit+crown FW (g)	452.2	483.7
Fruit DW (g)	40.5	25.4**
Crown DW (g)	7.8	24.4**
Fruit+crown DW (g)	48.3	49.8
Stem FW (g)	343.3	314.9
Stem DW (g)	82.3	44.6**
Peduncle FW (g)	145.8	68.1**
Peduncle DW (g)	16.1	5.8**
Leaf FW (g)	2239.9	2136.7
Leaf DW (g)	351.0	309.5
Total plant FW (g)	3181.2	3003.5
Total plant DW (g)	497.6	409.0*
Peduncle length (cm)	24.6	16.6**
Leaf area (m^2)	1.45	1.48
FN/Leaf area (cm^2)	70.0	49.15**
FN/Leaf FW	0.045	0.034*

† Data are means of 4 plants in the 30/20 °C treatment or 3 plants in 30/30 °C treatment.

‡ FW, fresh weight; DW, dry weight; FN, fruitlet number.

*, ** t-test significant at 0.05, 0.01 level of probability.

3.4 Discussion

Fruiting of pineapple is controlled by both internal and environmental factors. Temperatures have a wide spectrum of effects from metabolism at the molecular level to growth and development at a whole plant level. Because pineapple assimilates from 60 to 90% of its CO_2 at night, night temperature can significantly alter CO_2 dark fixation and affect assimilate supply (Bartholomew, 1982).

Growing pineapple plants 3 weeks at a 30 °C night temperature significantly decreased their susceptibility to floral induction with ethephon (Conway, 1977) . In our experiments, plants grown for 30 days or more in the 30/30 environment were forced with ethephon. The reason for the difference between the our results and those of Conway (1977) is not clear. However, in two of our experiments, some plants in the 30/20-30/30 treatment could not be forced with ethephon while plants from the same group in the 30/20 treatment were forced. Because plants in the 30/30 treatment were forced with ethephon, the forcing failure in the 30/20-30/30 treatment could not have been due to decreased ethephon uptake. Therefore, it is concluded that the result was due to decreased susceptibility of the plant to the growth regulator after the plants were transferred to the high night temperature environment.

The concept of sensitivity to a growth substance has been discussed thoroughly in recent publications (Trewavas, 1981; Trewavas, 1991; Trewavas and Cleland, 1983; Firn, 1986). This sensitivity is believed to be determined by receptor number and affinity for the growth regulator in question, and the "response capacity" of the plant (Firn, 1986). While the term sensitivity is commonly used to describe growth

responses, the term susceptibility has been widely used in the pineapple literature to describe the variable responses of plants to growth regulators used to induce flowering (Bartholomew and Kadzimin, 1977; Bartholomew and Criley, 1981; Py et al., 1987; Min and Bartholomew, 1993; see Appendix). It is clear that plant susceptibility, when used to describe the response of pineapple plants to flowering inductants, should include sensitivity to growth substances. Because of the difficulty in determining the receptor number (Sisler, 1991) and the inability to produce uniformly nonsusceptible plants, the internal factors controlling pineapple plant susceptibility to flower inductants were not examined. Further, the production of bracts but no flower structures on two of six plants in the 30/30 treatment in Experiment 3.1 is a result that supports the multifactorial control model of floral evocation proposed by Bernier et al. (1981b). The failure of plants to differentiate florets may be due to the absence of one or more growth regulators.

It was hypothesized that natural flowering of pineapple was controlled by naturally produced ethylene and to changes in plant susceptibility (sensitivity) to it (Min and Bartholomew, 1993; see Appendix). Plants grown at high night temperature did not flower naturally (Friend, 1981) and were difficult to force (Conway, 1977). The finding that plants grown at a 30 °C night temperature produced less ethylene and had lower ACCOase activity in stem and leaf tissue than did plants grown at a 20 °C night temperature, may account in part for the inhibitory effect of high night temperature on growth regulator-induced flowering of pineapple.

The reduced fruitlet number and fruit size for plants grown at high

temperatures are at least in part due to the reduced CO_2 dark fixation as indicated by the reduced leaf net TA for plants grown at those temperatures. The effect of temperature on the levels of plant hormones other than ethylene, or their metabolism, was not studied because there is virtually no information about their specific role(s) in the flowering process in pineapple.

CHAPTER 4

EFFECTS OF WATER STRESS ON ETHYLENE PRODUCTION, TITRATABLE ACIDITY, FLOWERING AND FRUIT DEVELOPMENT

4.1 Introduction

It is well-known that plants produce increased amounts of ethylene in response to stress. The research on the effects of excessive water and drought on ethylene production in plants has been comprehensively reviewed (Bradford and Yang, 1981; Yang and Hoffman, 1984; Abeles et al., 1992; Motto et al., 1992).

A number of plant species including trees, shrubs and herbaceous plants (Abeles et al., 1992) produced greater amounts of ethylene when subjected to water excess or anaerobic conditions. Ethylene production of tomato increased if the root system was waterlogged or exposed to low O_2. The increase was resulted mainly from increased production of 1-aminocyclopropane-1-carboxylic acid (ACC) in the roots, with subsequent transport of ACC to the shoot where it was converted to ethylene (Bradford and Yang, 1980; Wang and Arteca, 1991).

The effects of water deficit stress on ethylene production have been investigated using cotton bolls (Guinn, 1976), the petiole of cotton (McMichael et al., 1972), wheat leaves (Apelbaum and Yang, 1981), or intact plants of cotton, beans and miniature rose (Morgan et al., 1990) and wheat (Narayana et al., 1991). When detached organs lost moisture, they produced more ethylene than the unstressed

control (Apelbaum and Yang, 1981; Morgan et al., 1990; Narayana et al., 1991); however, no increase in ethylene production was detected from intact plants except cotton plants with a transient increase under water deficit stress (Morgan, et al., 1990; Narayana et al., 1991).

Pineapple is a xerophyte with a very high water use efficiency because it assimilates a large fraction of its carbon at night via Crassulacean acid metabolism (CAM) (Bartholomew and Kadzimin, 1977). Perhaps because pineapple was known to be tolerant to water stress, relatively few studies of the effects of water stress on vegetative growth have been conducted (Sideris and Krauss, 1928; Sideris and Krauss, 1955; Chapman et al., 1983) and relationships between soil and leaf water potential generally were not measured. Kadzimin (1975) examined the effects of the soil water potential on leaf water potential, leaf relative water content (RWC) and plant vegetative growth, and George et al. (1984) reported that the relationship between leaf water potential and RWC was linear and highly correlated. The literature on the effects of water stress on leaf gas exchange, plant growth, reproduction, and other physiological responses was comprehensively reviewed recently (Bartholomew and Malézieux, 1994); however, no study on the effects of water stress on ethylene production was reported. Although pineapple is tolerant to drought, irrigation increases yield while severe drought reduces it. In high rainfall regions, water excess also reduced plant productivity (Bartholomew and Malézieux, 1994). Chapman et al. (1983) found that less frequent watering of container-grown pineapple reduced the dry weight of all the parts of the plant, but fruitlet number was not affected.

Pineapple flowering can be induced with growth regulators such as ethylene, ethephon, and naphthaleneacetic acid (NAA) (Bartholomew and Criley, 1982; Py et al., 1987). It has been hypothesized that natural flowering of pineapple is induced by the endogenously produced ethylene, or by increased plant sensitivity (susceptibility) to ethylene, or both (Min and Bartholomew, 1993; see Appendix). Although pineapple was characterized as a quantitative short day plant (Gowing, 1961; Friend and Lydon, 1979), pineapple plants grown under nutrient or water stress or at a low night temperature tend to flower naturally and are highly susceptible to forcing agents (Bartholomew and Kadzimin, 1977).

No studies were found on the effects of water deficit and water excess stress during floral initiation on fruitlet number and fruit development of plants forced by a growth regulator. Fruit size is determined by fruitlet number and fresh weight per fruitlet, and both plant size at forcing and environmental factors after forcing influence fruit size and development (Py et al., 1987). In the report of Chapman et al. (1983), it was not possible to determine whether the reduction in fruit weight due to water stress resulted from a reduced plant size at forcing or reduced plant growth after forcing because watering treatments were begun at the time of planting and interim data on plant size and growth were not reported. Fruitlet number was reduced by a 30 °C post-forcing night temperature, which also reduced CO_2 dark fixation (Min and Bartholomew, 1995). Water stress may also influence floral initiation and reduce growth by decreasing CO_2 fixation.

The objectives of this study were to examine the effects of water excess and

water deficit stress on (1) ethylene production, leaf titratable acidity (TA) and natural flowering of pineapple; and (2) on leaf TA, and fruitlet number and fruit size of plants forced with ethephon.

4.2 Materials and Methods

Planting materials and treatments

In all the experiments only the 'Smooth Cayenne' pineapple clone Champaka F-153 was used.

Experiment 4.1 The effect of water excess on tissue ethylene production and natural flowering of pineapple was studied. Crowns weighing approximately 163 g (fresh weight) were planted on September 12, 1992 in 8.5 L (23.2 cm diameter by 20.0 cm high) pots using a medium consisting of half Sunshine #4 (a comercial mixture) and half horticultural perlite. Fertilizers [1.0 g Gaviota Foliar 62 (N 12%, P_2O_5 24%, K_2O 24%, Mg 0.04%, Fe 0.1%, Cu 0.013% B 0.01%, Mo 0.02%, Mn 0.012%, Zn 0.142%) (Brewer Environmental Industries, Honolulu), 0.04 g $CaCl_2$ and 0.3 g urea dissolved in 250 ml water per plant] were applied once every two weeks beginning one month after planting and plants were irrigated weekly with tap water. The plants were grown in a glasshouse.

Because pineapple tends to flower naturally during the winter in Hawaii in response to short daylength and low temperature, to avoid any confounding effect of natural induction, plants were treated with excessive water in April, 1993. Plants in pots were each put in partially sealed pots to slow down water drainage. To have the

water excess treatments end on the same day, plants were watered in the morning, noon and afternoon of each day for 14, 10, 6, 3, and 1 days before sampling. Control plants were watered weekly, the last time being 3 days before sampling. D-leaf basal white (white) and stem apical (stem) tissues were sampled from three plants from each treatment for the measurement of ethylene and ACC oxidase (ACCOase) activity on April 30. Ten plants each in the control and 14-day treatments were maintained to observe natural flowering. Fertilization was stopped at the time the treatments were begun. The time of inflorescence appearance was recorded when the inflorescence was visible in the leaf whorl and about 1.0 cm in diameter in all experiments.

Experiment 4.2 To further examine the effects of waterlogging on tissue ethylene production and pineapple natural flowering, crowns weighing 125 g each were planted in pots on May 31, 1993. The maintenance of plants was as described in Experiment 4.1. Plants were waterlogged from April 27, 1994 (14 days), May 4 (7 days), or May 9 (2 days) until May 11 by keeping the water level 2 cm above the media surface of pots placed in another pot that was lined with a plastic bag. The control plants were watered weekly. There were 8 plants in each treatment. Plants were sampled on May 11, 1994 for the measurement of leaf relative water content (RWC), leaf TA, ethylene production, and ACCOase activity of leaf green and white and stem apical tissues.

After the treatments were ended, plants were grown out-of-doors to observe natural flowering. Leaf TA and leaf RWC were measured 14, 35 and 57 days after

the treatments were ended. Fertilization was stopped at the time the treatments were begun and resumed 3 months after the treatments were ended.

Experiment 4.3 The effects of water deficit stress on leaf and stem ethylene production, forcing response, and natural flowering of pineapple were studied in this experiment. Crowns weighing approximately 125 g each were planted in pots on June 1, 1993 and the plants were maintained as in Experiment 4.1. Watering was stopped from April 27 to June 22, 1994 (8 weeks) and from May 18 to June 22, 1994 (5 weeks), respectively, while control plants were watered weekly. There were 12 plants in each treatment. During water stress, no fertilizer was applied. The leaf RWC, leaf TA, ethylene production, and ACCOase activity of leaf green and white and stem apical tissues were measured on June 23. Plants were moved from the glass-house to a field site out-of-doors after the treatments were ended. After re-watering, four plants from each treatment were treated with ethephon (10 mg in 10 ml of a 2% urea-water solution per plant) to induce flowering. Leaf TA and RWC were measured after forcing, and a second application of ethephon was made on August 26 (64 days after the first application) to plants not forced by the first treatment. Four plants were held to observe natural flowering. No fertilizers were applied during the water stress treatment. Fertilizer was applied once per two weeks to ethephon-forced plants. No fertilizer was applied to plants held to observe natural flowering for 3 months after treatment, after which regular fertilization was resumed.

Experiment 4.4. This experiment was designed to examine the effects of water deficit and water excess stress during the period of floral initiation on fruitlet

number and fruit development. Crowns having fresh weights of about 175 g were planted in pots on April 25, 1993 and the plants were maintained in a glass-house as described in Experiment 4.1. Plants were watered once per ten days after planting.

Twenty five plants were selected for this experiment. There were 5 treatments with 5 plants each. These treatments were (1) control-plants, which were watered once per 10 days; (2) withholding water for 3 weeks and then re-watering (d-1); (3) withholding water for 6 weeks and then re-watering (d-2); (4) waterlogging for 3 weeks and then draining excessive water (w-1); (5) waterlogging for 6 weeks and then draining excessive water (w-2). Waterlogging was carried out by putting the potted plant into another pot lined with a plastic bag to prevent water drainage. The water level was kept slightly above the medium surface. The treatments were begun on February 23, 1994, 16 days from the last watering and all plants were treated to initiate flowering on that day by pouring 10 ml of a 2% urea-water solution containing 10 mg ethephon into the center of each plant. No fertilizer was applied during the period of water stress. Fertilization was resumed 80 days after the treatments were begun.

Leaf RWC and TA were measured weekly, first on the D-leaf and then on the younger leaves. Fruits were harvested when a fruit was one-third yellow. The date of harvesting, fruitlet number, fruit fresh and dry weight, leaf area, and leaf, peduncle and stem dry weight were recorded at harvesting.

Measurement of ethylene and ACCOase activity

Ethylene production was assayed by placing about 1.0 g of leaf or stem apical

tissue into a 17.5 ml test tube, the tube was sealed with a serum stopper, and the sample was incubated at about 25 °C in room light for 2 or more hours. After incubation, ethylene production was measured by withdrawing a 1.0 ml gas sample from the headspace and injecting it into a gas chromatograph (GC) equipped with a flame ionization detector. For the measurement of ACCOase activity, about 1.0 g fresh leaf or stem tissue was put into a 1.0 mM ACC-water solution for 2 minutes, blotted dry, placed in a 17.5 ml test tube, sealed with a serum stopper, and incubated for 2 or more hours as described above. Ethylene was then measured by the GC (Min and Bartholomew, 1993; see Appendix).

Determination of leaf RWC and leaf TA

Leaf discs (about 2 cm^2) were sampled from the middle part of the D-leaf or subsequent younger leaves. For the determination of TA, leaves were sampled both in the early morning before sunrise (am) and in the evening just after sunset (pm). For RWC measurement, leaves were sampled only in the early morning. To measure TA, two leaf discs were ground in a mortar and brought to about 50 ml with deionized water, and boiled for 10 minutes. After cooling, the sample was titrated with 0.01 N NaOH to a pH 8.0 end point (Modified from Friend and Lydon, 1979).

Leaf RWC was measured following the procedure of Barrs and Weatherley (1962) and George et al. (1984). After obtaining the fresh weight (FW) of a leaf disc, it was floated on deionized water in a petri dish for 4 hours at room temperature (25°C) and light (10 $\mu M\ m^{-2}\ s^{-1}$). The discs were blotted dry and reweighed to obtain the turgid weight (TW), then oven dried for 24 hours at 70 °C. After measuring the

dry weight (DW), RWC was calculated by the formula:

RWC (%) = (FW-DW)/(TW-DW)*100%.

4.3 Results

4.3.1. Effects of water deficit and water excess stress on tissue ethylene production

Ethylene production in D-leaf basal-white tissue increased with increasing treatment time and was significantly greater than the control after 10 and 14 days of excessive water treatment (Experiment 4.1) (Figure 4.1). There was a transient increase in ethylene production by stem apical tissue after one day of treatment, but the difference among treatments was not significant (Figure 4.1). The activity of ACCOase in D-leaf basal-white tissue increased steadily as duration of the treatment increased, and was significantly greater than in the control after 14 days of treatment. In stem apical tissue, ACCOase activity remained unchanged over the duration of the treatment and no significant effect due to treatment was found (Figure 4.1).

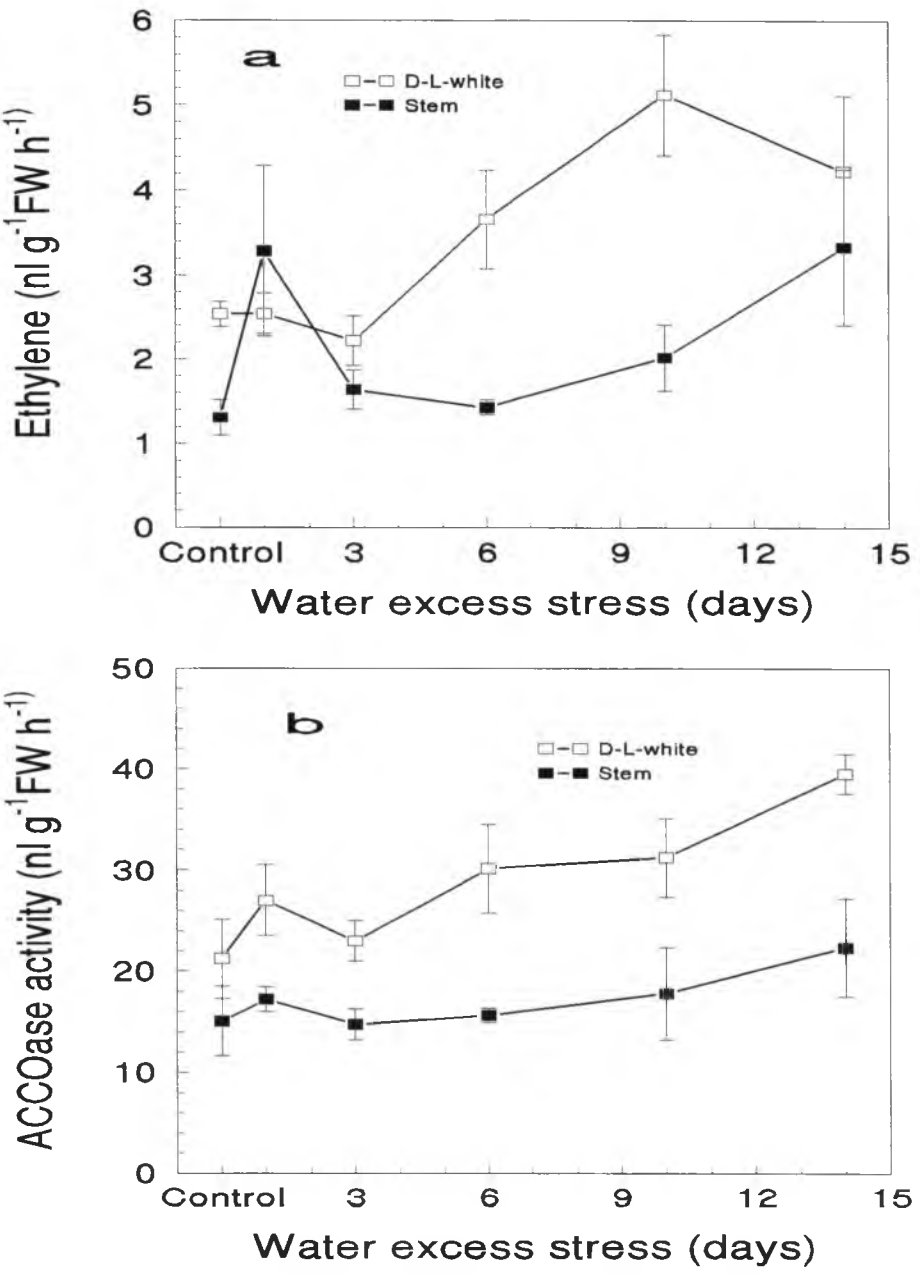

Figure 4.1. Effects of water excess stress on ethylene production (a) and 1-aminocyclopropane-carboxylic acid oxidase (ACCOase) activity (b) of pineapple D-leaf basal-white (D-L-white) and stem apical (Stem) tissues (Experiment 4.1). Tissues were incubated for 3 hours 5 minutes. Data are means of 3 replicates $\pm$ SE. Values of LSD (0.05) are 1.69 for ethylene production of D-leaf basal-white tissue, and 10.46 for ACCOase activity of D-leaf basal-white tissue. There were no significant differences among treatments in ethylene production and ACCOase activity of stem apical tissue by the F-test.

With complete waterlogging (Experiment 4.2), there were no significant effects of treatment on ethylene production in any of the tissues (Table 4.1). ACCOase activity in the leaf green and basal white tissues was not affected by treatment, but it was significantly reduced in stem apical tissue after 14 days of treatment relative to the control (Table 4.1).

Table 4.1. Effects of waterlogging on ethylene production and ACC oxidase (ACCOase) activity of pineapple D-leaf green (G), D-leaf basal white (W) and stem apical (S) tissues (Experiment 4.2).

	Days of treatment				
	Control	2	7	14	LSD (0.05)
	- - - - - - - - - - - - nl $gFW^{-1}h^{-1}$ - - - - - - - - - - - -				
G-ethylene†	0.57‡	0.98	0.64	0.61	ns
W-ethylene	6.30	5.71	6.91	4.38	ns
S-ethylene	4.93	4.41	5.16	2.03	ns
G-ACCOase	2.80	2.68	2.15	1.98	ns
W-ACCOase	116.92	158.67	123.07	125.38	ns
S-ACCOase	43.10	45.27	37.51	34.39	7.25

† Tissues were incubated for 4.5 hours.
‡ All values are means of data from 3 plants; ns is not significant at the 0.05 level of probability by F-test.

In Experiment 4.3, water deficit stress as a result of withholding water for 35 and 56 days had no effect on ethylene production by leaf green or stem apical tissue. Ethylene production by leaf basal white tissue decreased significantly after withholding water for 35 and 56 days (Table 4.2). ACCOase activity in green tissue also remained unchanged, while ACCOase activity in leaf basal white and stem apical

tissues was significantly reduced after 35 and 56 days of stress. The differences between 35 and 56 days of treatment were not significant (Table 4.2).

Table 4.2. Effects of water deficit stress on ethylene production and ACC oxidase (ACCOase) activity of pineapple D-leaf green (G), D-leaf basal-white (W) and stem apical (S) tissues (Experiment 4.3).

	Days of treatment			
	Control	35	56	LSD (0.05)
	- - - - - - - - nl $gFW^{-1}h^{-1}$ - - - - - - - -			
G-ethylene†	0.35‡	0.28	0.29	ns
W-ethylene	3.20	0.66	0.53	1.13
S-ethylene	0.63	0.46	0.46	ns
G-ACCOase	1.52	0.94	1.07	ns
W-ACCOase	51.04	17.62	2.32	33.31
S-ACCOase	28.39	6.68	2.47	12.97

† Plant tissues were incubated for 2.5 hours.
‡ Values are means of data from 3 plants; ns not significant at the 0.05 level of probability by the F-test.

4.3.2. Effects of water deficit and water excess stress on leaf RWC and TA

Leaf RWC decreased significantly with increasing time as a result of waterlogging for more than 2 days (Experiment 4.2, Figure 4.2a). Along with the decrease in RWC, the leaf am TA (Figure 4.2b) and net TA (Figure 4.2d) also decreased significantly after 7 and 14 days of treatment but not after 2 days. Leaf pm TA was significantly decreased by waterlogging treatment (Figure 4.2c).

After the water was drained, leaf RWC and am TA of waterlogged plants gradually recovered. At 35 days after the water was drained, leaf RWC, am and net TA in the 7-day treatment were not significantly different from the control. At 57

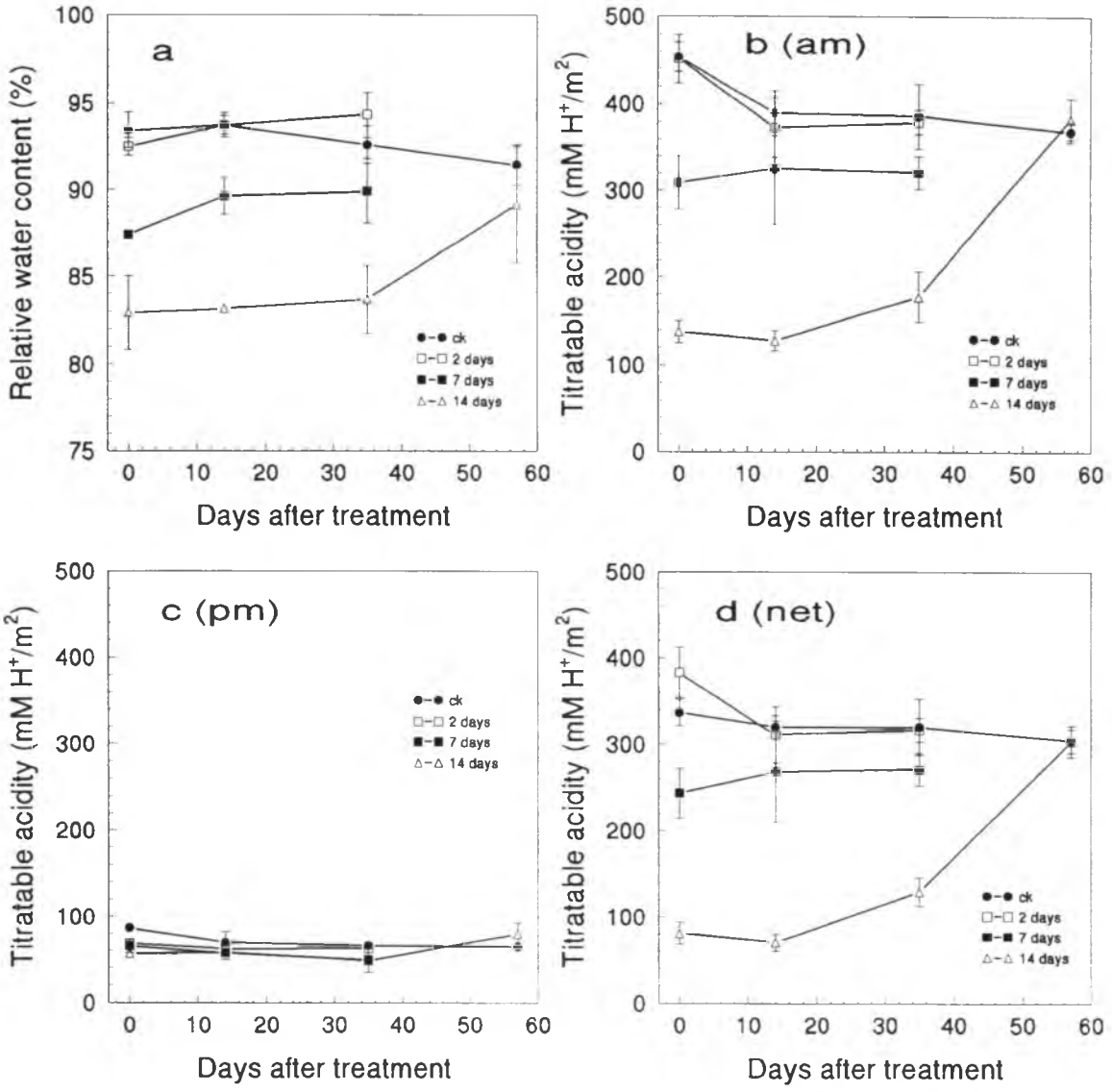

Figure 4.2. Effects of waterlogging for 0, 2, 7, and 14 days on leaf relative water content (a) and leaf am (b), pm (c) and net (d) titratable acidity of pineapple (Experiment 4.2). Imposition of the treatments was staggered and the first sampling was made at zero days after treatments were ended. Values are means of 3 plants ± SE.

days after the water was drained, leaf RWC, am and net TA in the 14-day treatment recovered to the control level (Figure 4.2 a, b, d). The leaf pm TA level was not significantly different among treatments after water drainage (Figure 4.2c).

Water deficit stress (Experiment 4.3) significantly decreased leaf RWC (Figure 4.3a), leaf am TA (Figure 4.3b), and net TA (Figure 4.3d), while pm TA (Figure 4.3c) was not affected. After re-watering plants, leaf RWC, am and net TA recovered within 7 days in the 35-day water stress treatment. The leaf RWC in the 56-day treatment still was significantly lower than the control 14 days after plants were re-watered while values of leaf am and net TA reached the control levels.

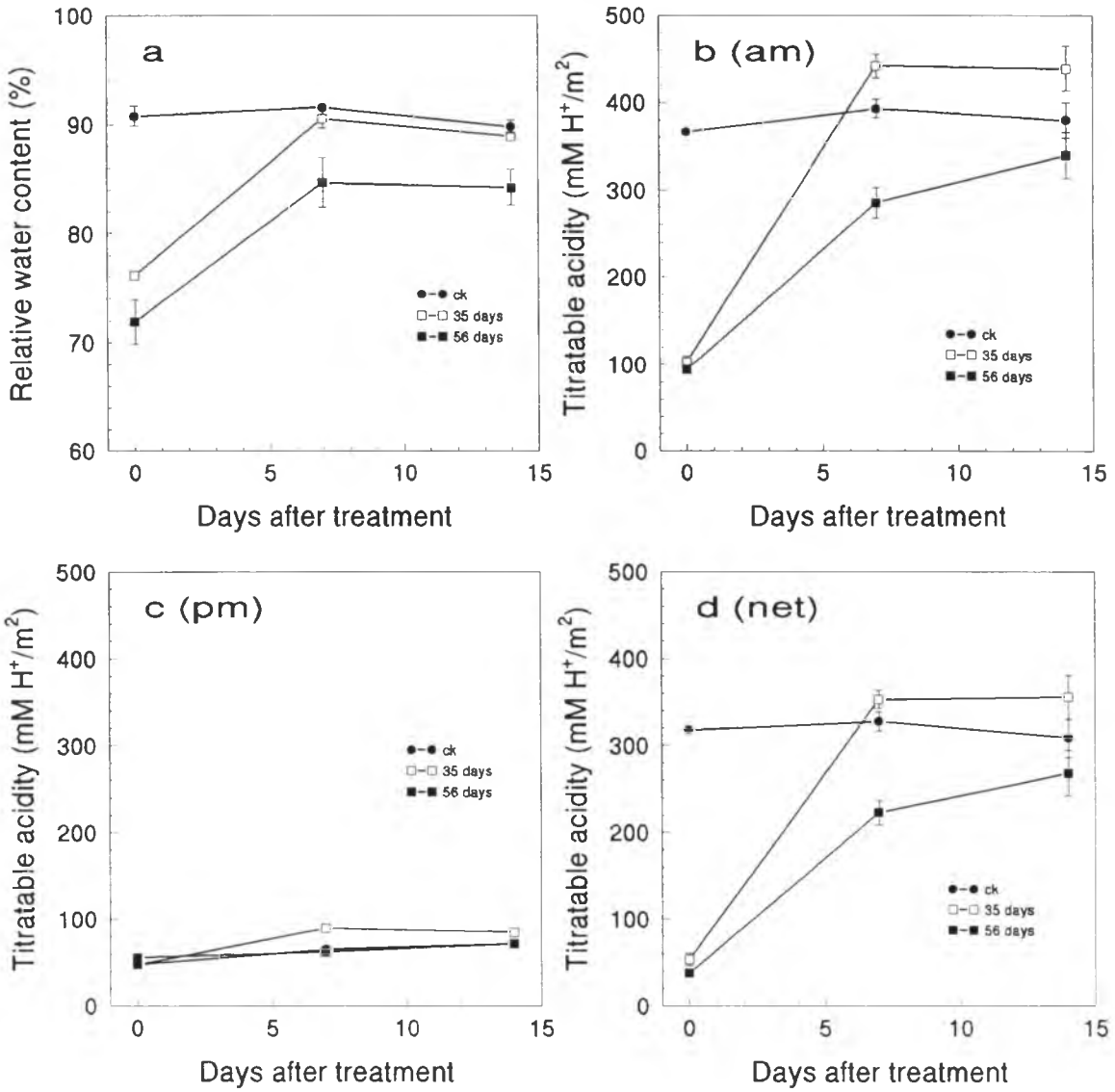

Figure 4.3. Effects of water deficit stress on leaf relative water content (a) and leaf am (b), pm (c), and net (d) titratable acidity of pineapple (Experiment 4.3). Imposition of the treatments was staggered and the first sampling was made before plants were re-watered on day zero. The second and third samplings were made, respectively, 7 and 14 days after the plants were re-watered.

In a more detailed study (Experiment 4.4) of the effects of waterlogging and water deficit stress on leaf RWC and leaf TA, similar results were obtained (Figure 4.4). Both waterlogging and water deficit stress decreased leaf RWC, and am and net TA (Fig. 4.4 a, b, d). As was previously observed, there was little difference in the pm TA levels among treatments (Fig. 4.4 c). After draining waterlogged plants and re-watering the water deficit plants, recovery from the treatment effects was followed. For the 3-week (d-1) water deficit treatment, both leaf RWC and TA recovered very rapidly after re-watering and more slowly in the 6 week (d-2) treatment. Plant response to waterlogging for 3 (w-1) and 6 (w-2) weeks were similar. After the water was drained, both leaf RWC and TA recovered very slowly. The increase in leaf pm TA at the week 4 sampling could have been caused by continuous cloudiness and rainy weather during that period.

There was a significant positive linear relationship between leaf RWC and leaf am TA (Figure 4.5a). Physiologically, there was no significant effect of leaf RWC on leaf pm TA (Figure 4.5b). The net TA was also highly correlated with leaf RWC (Figure 4.5c).

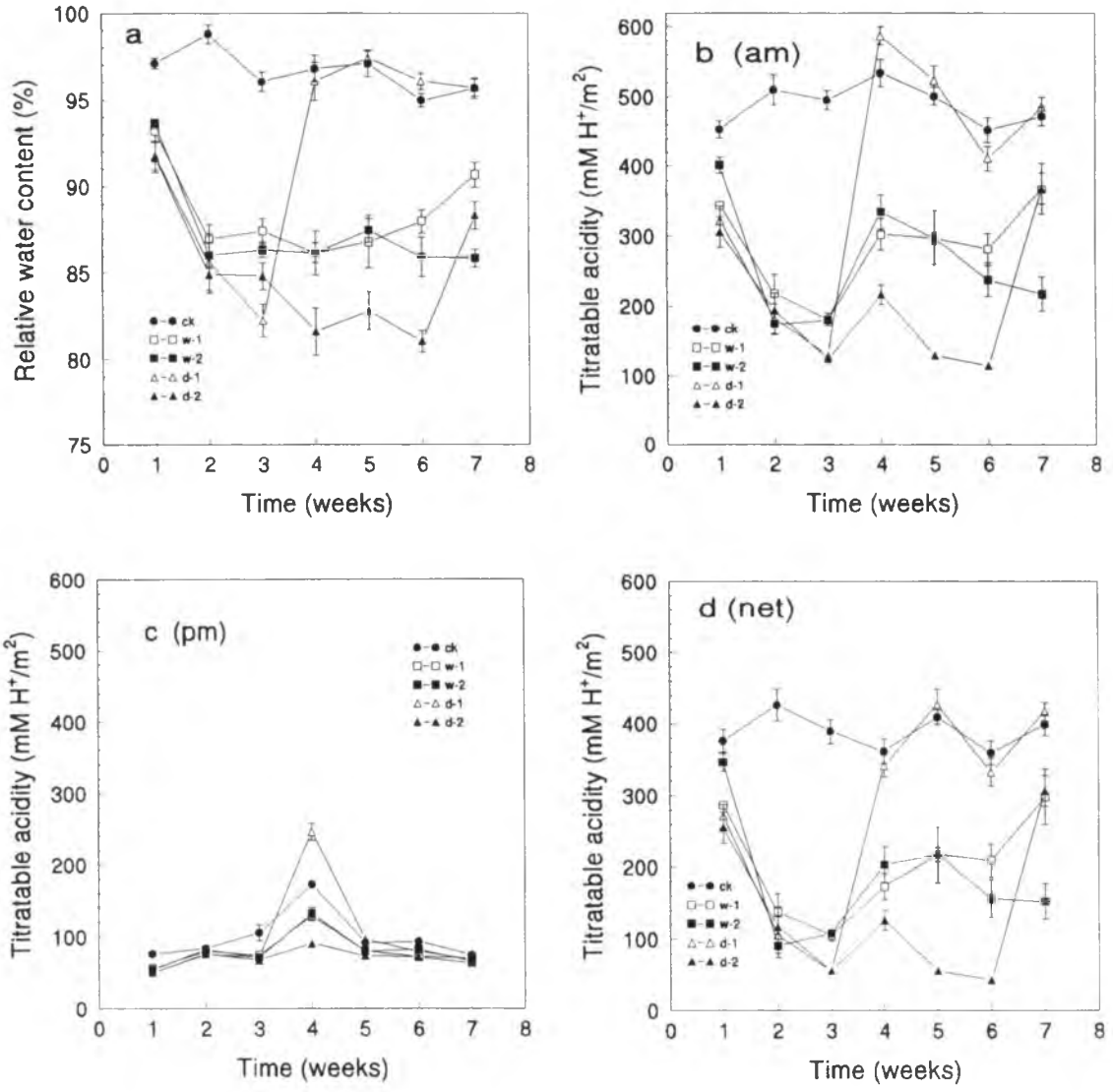

Figure 4.4. Effects of waterlogging and water deficit stress on leaf relative water content (a), leaf am (b), pm (c), and net (d) titratable acidity. The treatments were control (ck), which was watered every 10 days; plants waterlogged for 3 (w-1) or 6 (w-2) weeks, after which the water was drained; and plants stressed by withholding water for 3 (d-1) or 6 (d-2) weeks. The treatments were started from week 0. All plants were watered as the control after removing the stress.

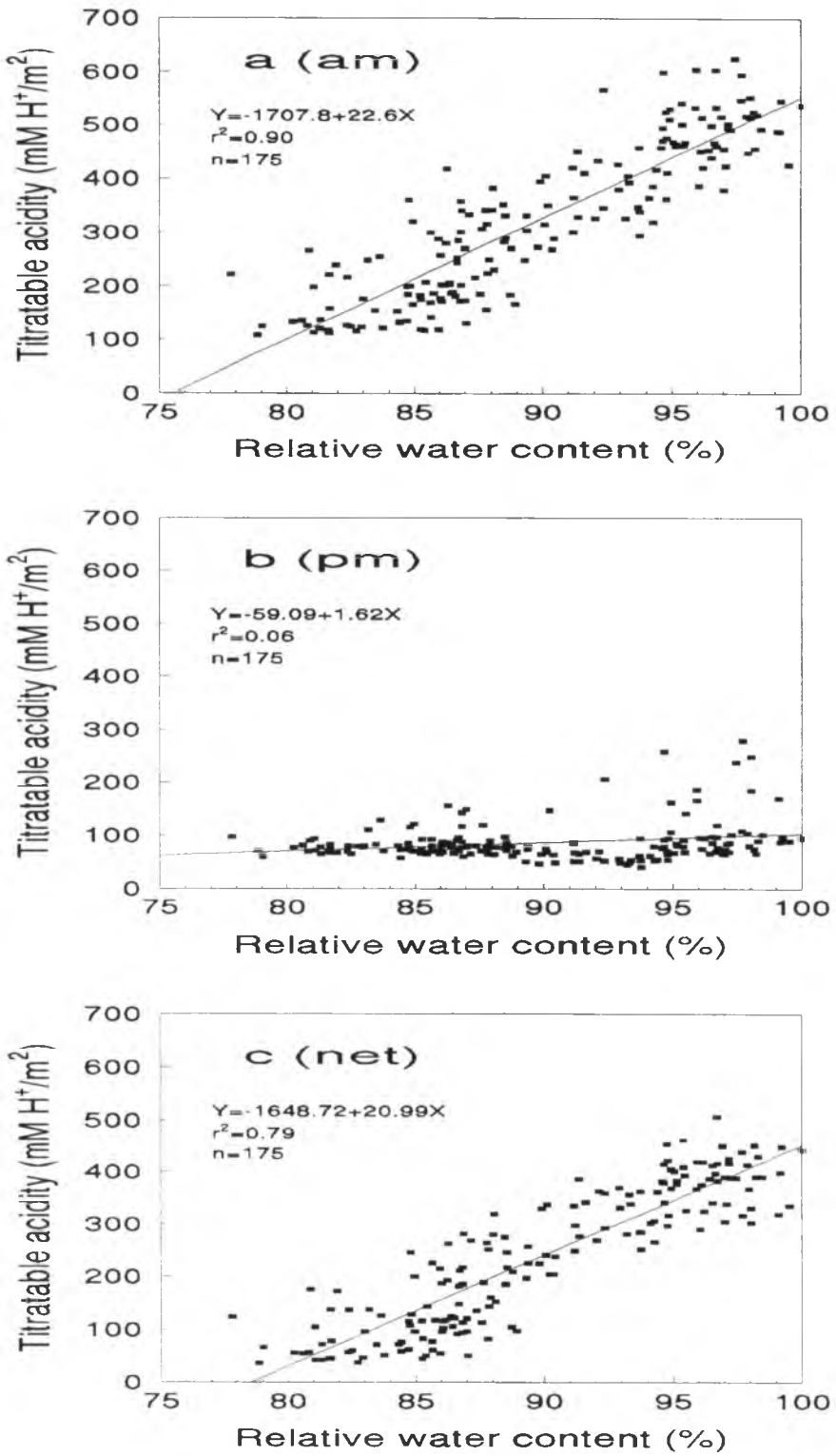

Figure 4.5. Relationships between leaf relative water content and leaf am (a), pm (b), and net (c) titratable acidity.

4.3.3. Effects of waterlogging and water deficit stress on pineapple floral initiation and development

Water excess stress did not induce plants to flower as no inflorescences appeared within 2 months after treatment (Experiment 4.1, 4.2). In Experiment 4.1, the control plants and those exposed to water excess stress for 14 days flowered simultaneously in the spring of the following year (about 10 months later). In Experiment 4.2, the inflorescences of the control and waterlogged plants appeared between 2 and 9 months after the treatments were imposed (from July, 1994 to January, 1995) (Table 4.3). There were no significant treatment effects on the days from end of treatments to the appearance of the inflorescence and these plants were not induced by waterlogging stress.

Table 4.3 Effect of waterlogging on days from the end of the stress treatment to appearance of naturally induced inflorescences of pineapple (Experiment 4.2, n=5)†.

	Days of treatment		
Control	2	7	14
71	64	130	115
138	132	131	226
173	132	131	240
240	141	155	240
254	246	246	246

† The waterlogging treatments were ended on May 2, 1994.

Water deficit stress decreased plant susceptibility to ethephon forcing (Experiment 4.3, Table 4.4). All the control plants (one flowered naturally) and two of four plants water stressed for 56 days were induced to flower by ethephon applied

immediately after plants re-watered. None of the plants in the 35-day water stress treatment were forced by the first application of ethephon. However, all the remaining unforced plants were induced to flower by the second application of ethephon made 64 days after re-watering (Table 4.4).

Table 4.4 Effect of water deficit stress on days to appearance of pineapple inflorescences after forcing with ethephon (Experiment 4.3, n=4).

	Days of treatment	
Control	35	56
12†	103*	44
44	103*	52
47	103*	103*
50	104*	107*

† Days from the first application of ethephon to inflorescence appearance.
* Plants were not forced by the first application of ethephon and forced by the second ethephon application, which was made 64 days after first one.

Table 4.5 Effect of water deficit stress on days from the end of the stress treatment to appearance of naturally induced pineapple inflorescences (Experiment 4.3, n=4)

	Days of treatment†	
Control	35	56
22	57	195
195	195	196
195	198	197
196	198	202

† Water was withheld for 35 and 56 days, after which plants were re-watered (on June 23, 1995).

Water deficit stress did not directly induce natural flowering of pineapple (Experiment 4.3, Table 4.5). One control plant and one plant in the 35-day water deficit treatment that flowered within the first two months after treatment (22 days for the control and 57 days for the 35-day treatment). The inflorescences of other plants appeared in January, 1995, approximately 7 months after the treatments had been imposed. The January flowering was assumed to be in response to the short daylengths and low temperatures prevailing during the winter season (Table 4.5).

In Experiment 4.4 (Table 4.6), waterlogging was imposed or water was withheld from 10-month old plants immediately following ethephon treatment on February 23, 1994. The inflorescences of forced plants usually appear about two months after treatment (Bartholomew, 1977). The experiment was carried out during the winter season and some plants initiated flower development naturally, as indicated by the appearance of inflorescences on some plants within one month after forcing; these plants were discarded. One plant in the control was not forced for unknown reasons, and that plant flowered the following August. Therefore, there were 3 plants in the control, and 5 plants each in the 3- (d-1) and 6- (d-2) week water deficit treatments, and 3 plants in the 3- (w-1) and 4 plants in the 6- (w-2) week waterlogging treatments used in the analysis (Table 4.9). The time from forcing to inflorescence appearance (IA) was about 50 days for the control, w-1,w-2, and d-1 treatments. The inflorescence appeared an average of 16 days later in the d-2 treatment than in the control. Duration from forcing to fruit ripening (FD) was similar for the control, d-1, and w-1 treatments, while the duration for the w-2 and d-

Table 4.6. Effects of water deficit for 3 (d-1) and 6 weeks (d-2) or waterlogging for 3 (w-1) and 6 weeks (w-2) on floral initiation and development of pineapple forced by ethephon (Exp. 4.4).

Parameters	Control	d-1	d-2	w-1	w-2
Total plants	5	5	5	5	5
Forced plants†	3	5	5	3	4
IA‡ (days)	50b§	49b	66a	48b	53b
FD (days)	196b	198b	211a	201b	213a
Fruitlet number	94a	73a	44b	74a	68ab
Fruit FW (g)	1458.6a	1380.9a	944.0b	926.3b	717.5b
Fruit DW (g)	192.2a	196.2a	130.1b	127.5b	114.0b
Fruitlet FW (g)	15.5bc	18.8ab	22.4a	13.1cd	10.9d
Fruitlet DW (g)	2.0bc	2.7ab	3.1a	1.8c	1.4c
Crown FW (g)	617.3	703.6	840.7	705.6	733.6
Crown DW (g)	84.8	95.9	106.5	106.0	113.4
F+C FW (g)	2075.9a	2084.4a	1784.7ab	1631.9b	1451.1b
Peduncle (cm)	22.3	20.0	18.8	16.7	13.4
Peduncle DW (g)	10.5a	9.9a	7.8b	6.1b	4.6c
Leaf area (cm^2)	10725.0	10615.8	8764.4	8989.9	8925.8
Leaf DW (g)	209.9	207.1	174.6	189.3	190.1
Dead leaf DW (g)	27.1ab	22.5b	37.3a	20.6b	29.4ab
Stem DW (g)	46.5	50.3	44.0	51.6	53.8
Total DW (g)	571.0	581.8	500.3	501.1	505.3
FN/LA (m^{-2})	89.0a	69.0ab	50.0b	81.0a	78.0a
FDW/TDW (%)	33.6ab	33.7a	25.5bc	25.3 bc	23.3c

† Some plants initiated an inflorescence prior to ethephon forcing, and these plants were excluded. There was one plant in the control unforced.

‡ Abbreviations are IA, days from ethephon treatment to inflorescence appearance; FD, days from forcing to fruit harvest; FW, fresh weight; DW, dry weight; F+C, fruit plus crown; FN/LA, fruitlet number/leaf area (m^2); FDW/TDW, fruit dry weight/total dry weight.

§ Means in the same row followed by the same letter or no letter are not significantly different at the 0.05 level of probability by the LSD test.

2 treatments was delayed by about two weeks relative to the control. The delay in the d-2 treatment could have been due to the fact that the inflorescence appeared late but the reason for the delay in the w-2 treatment is not known.

Fruitlet number was significantly reduced in the d-2 treatment relative to the control, while there was no significant difference between the control and the d-1 treatment. Waterlogging had no significant effect on fruitlet number. There was no significant difference in fruit fresh and dry weights between control and the d-1 treatment, while these weights in the d-2 treatment were significantly less than the control. Fruitlet fresh and dry weights were significantly greater in the d-2 treatment than in the control. Fruit fresh and dry weights in the w-2 treatment were significantly less than the control because of a significant decrease in fruitlet fresh weight. However, waterlogging did not significantly affect fruitlet dry weight. Crown fresh and dry weights were similar among the treatments. Peduncle dry weight was significantly reduced in the d-2 treatment and in both waterlogging treatments while the length of peduncle was similar among treatments.

Some treatment differences could have been caused by differences in leaf area among the treatments at forcing. However, since leaf area is not easily measured nondestructively, the leaf area at the time of fruit maturity was used in lieu of the leaf area at forcing. There were no significant differences in leaf area or leaf dry weight among the treatments. There were relatively more dead leaves in the d-2 and w-2 treatments than in the control at the time of fruit maturity. The ratio of fruitlet number to total leaf area was calculated to estimate the efficiency of leaves in

supplying carbohydrate to the developing fruit. There were no significant differences in the ratio among the control, d-1, w-1 and w-2 treatments. The value for the d-2 treatment was significantly less than the control but not different from that for treatment d-1.

There were no significant differences in stem or total plant (excluding roots) dry weight among treatments. The ratio of fruit dry weight to total plant dry weight, a measure of dry matter partitioning, was significantly less for the w-2 treatment than for the control and d-1 treatments. The ratio for the d-2 and w-1 treatments were significantly less than for the d-1 treatment.

4.4 Discussion

Water stress affects a wide spectrum of plant processes including hormones, metabolism, translocation, transpiration and stomatal conductance, plant growth and morphology (Hsiao, 1973). Few data appear to have been collected on the effects of water deficit or waterlogging stress on ethylene production of CAM plants. The CAM-plant *Kalanchoe daigremontana* had increased ethylene production in response to the imposition of waterlogging or drought (Kapuya and Hall, 1984). No other published study on this subject was found.

In the first trial (Experiment 4.1) where the response of pineapple to water excess stress was examined, ethylene production and ACCOase activity in the leaf basal tissue were increased in response to water excess stress whereas they remained unchanged in the completely waterlogged trial (Experiment 4.2). The differences in

ethylene production and ACCOase activity in these two experiments could be due to the differences in the treatments. In the first experiment, the plants were not waterlogged because pots were not completely sealed, whereas in the second experiment, the treated plants were completely waterlogged. The possibility of a transient increase in ethylene production in both stem and leaf tissues in Experiment 4.2 cannot be excluded, because there was a large time lapse between samplings. The results of the first trial are consistent with results obtained by Kapuya and Hall (1984) for *Kalanchoe daigremontana* and other results obtained with mesophytic plants (Bradford and Yang, 1980; Kawase, 1975). In neither experiment did water excess promote natural flowering.

Fluctuation in water availability is apparently an essential factor in the control of flower formation in some plants (Bernier et al., 1981a). A period of water shortage is absolutely required for flower initiation of *Geophila renaris* (Bernier et al., 1981a). Water stress promoted growth of floral buds in mango (Nunez-Elisea and Davenport, 1994) and increased flowering intensity and yield in litchi (Stern et al., 1993). Water stress increased leaf ethylene production and broke the flower bud dormancy in coffee (Schuch et al., 1992).

The significant decrease in leaf RWC and am TA in water deficit stressed plants indicated the plants were under severe stress. Water deficit stress decreased ethylene production and ACCOase activity in pineapple leaf basal tissue, and reduced susceptibility to ethephon forcing in this experiment. This result seems to contradict the existing dogma that plants are more susceptible to forcing under limited water

supply (Bartholomew and Kadzimin, 1977; Py et al. 1987). However, in this experiment, the potted plants lost water very rapidly as indicated by the sharp decline in leaf RWC, and the growth of plants might have been stopped. The conditions of potted plants with limited soil volume are quite different from those of plants grown in a field where water stress would increase gradually and the roots of plants would be able to grow deeper in the soil to exploit water.

Because both water deficit and waterlogging stress did not induce pineapple flowering, it is concluded that water does not play an important role in the control of pineapple floral initiation. However, these experiments were carried out with plants grown in pots and it is difficult to extrapolate the above conclusion to a field situation.

Both water deficit and waterlogging stress significantly decreased leaf dark CO_2 fixation as indicated by leaf am TA. Leaf am and net TA were highly and positively correlated with leaf RWC. George et al. (1984) reported that leaf water potential (ψ) and leaf RWC were linearly correlated and leaf RWC was a reasonably sensitive measure of ψ for pineapple. In experiments reported here, leaf RWC and TA were measured simultaneously and were highly correlated in both waterlogged and water deficit stressed plants. These results indicate that both leaf RWC and TA, two easily measurable parameters, are useful when examining the physiological status of pineapple plants under water stress. The changes in leaf RWC and TA, the correlation between leaf RWC and TA, and the recovery after re-watering of pineapple plants under water deficit stress were basically similar with Bastide et al.

(1993) on *Xerosicyos*, a CAM plant, under water deficit stress.

Although water stress did not influence pineapple natural flowering, water stress reduced pineapple fruitlet number, fruit size, and delayed fruit ripening when pineapple flowering was forced with ethephon. Chapman et al. (1983) found that watering frequencies did not influence fruitlet number; however, fruit fresh weight in the treatment watered once per two months was only one-fifth the fresh weight of the fruit in the treatment that was watered twice weekly. Our results showed that post-forcing water status was a very important factor controlling the fruitlet number. The reduced fruitlet number resulted from the decreased CO_2 dark, and presumably total, fixation.

CHAPTER 5

EFFECTS OF GROWTH REGULATORS ON ETHYLENE PRODUCTION AND FLOWERING

5.1 Introduction

Pineapple is an important fruit crop in the tropics and subtropics. Inducing flowering (forcing) with a growth regulator such as ethylene, ethephon or naphthalene acetic acid (NAA) to synchronize fruit initiation is a common practice on most modern farms and plantations (Bartholomew and Criley, 1983; Williams, 1987). However, unexpected precocious flowering prior to the planned forcing is a widespread problem in most pineapple producing regions because it disrupts crop scheduling and produces fruits that are either too small to be marketable or too few which makes harvesting unprofitable (Bartholomew and Malézieux, 1994; Scott, 1993).

Numerous attempts have been made to find chemicals that will delay natural flowering of pineapple. Leeper (1965) and Millar-Watt (1981) reported that large amounts of NAA inhibited flowering, but it has never been used commercially because it is a forcing agent when applied in smaller amounts. Millar-Watt (1981) reported that three applications of silver nitrate at one-month intervals between March and May decreased the natural flowering percentage from 57 to 27. Scott (1993) reported that 2-(3-chlorophenoxy)propionic acid (Fruitone CPA, Fruitone) reduced the

percentage of precocious fruit from 48.5 to 8.2 in a ratoon field because sucker growth was inhibited. Silver ion, an inhibitor of ethylene action (Abeles et al., 1992), applied as silver nitrate or silver thiosulfate (STS) blocked floral induction of pineapple by ethephon (Sanford and Bartholomew, 1981; Min and Bartholomew, 1993; see Appendix).

It has been hypothesized that natural flowering of pineapple is induced by either naturally produced ethylene or increased plant susceptibility (sensitivity) to ethylene, or both (Min and Bartholomew, 1993; see Appendix). Because the physiological basis of plant susceptibility is not known, attempts to inhibit or delay pineapple natural flowering have involved testing compounds that can inhibit plant ethylene biosynthesis or ethylene action. In the research reported here, compounds that inhibit ethylene biosynthesis, such as aminooxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) (Yang and Hoffman, 1984), and inhibitors of ethylene action such as silver ion (silver thiosulfate, STS), were evaluated. Also, evaluated were growth regulators that decrease plant tissue ethylene production, but do not directly inhibit 1-aminocyclopropane-1-carboxylic acid (ACC) synthase or ACC oxidase (ACCOase; or ethylene forming enzyme, EFE), such as uniconazole (Kraus et al., 1991), paclobutrazol (Wang and Steffens, 1985) and daminozide (Gussman et al., 1993). Gibberellic acid (GA_3) and Fruitone were also included in different experiments to evaluate their effects on natural flowering. To examine the possible modes of action of some of the above growth regulators on pineapple flowering, their effects on ethylene production, ACCOase activity, and ACC and 1-

malonylaminocyclopropane-1-carboxylic acid (MACC) content in plant tissues were measured.

5.2 Materials and Methods

Plant materials and treatments

In all the experiments only the 'Smooth Cayenne' pineapple clone Champaka F-153 was used. Each plant was grown in an 8.5 L (23.2 cm diameter by 20.0 cm high) pot with a 1:1 (by volume) mixture of Sunshine #4 (a commercial mixture) and horticultural perlite. Each plant was watered weekly and fertilized once per two weeks with 1.0 g Gaviota Foliar 62 (N 12%, P_2O_5 24%, K_2O 24%, Mg 0.04%, Fe 0.1%, Cu 0.013% B 0.01%, Mo 0.02%, Mn 0.012%, Zn 0.142%) (Brewer Environmental Industries, Honolulu), a commercial soluble fertilizer, 0.04 g $CaCl_2$ and 0.3 g urea dissolved in 250 ml water beginning one month after planting. The time of inflorescence appearance was recorded when the inflorescence was visible in the leaf whorl and about 1.0 cm in diameter in all the experiments.

Experiment 5.1 Suckers weighing about 325 g were planted on March 5, 1992. Plants were grown out-of-doors for 161 days. Plants were treated with either 20 mg GA_3 or 10 mg uniconazole by pouring 10 ml of a water solution containing the growth regulator into the center of each plant on August 13, 1992 (161 days after planting). Deionized water was used as the control. There were 16 plants in the control, and 17 plants each were treated with GA_3 or uniconazole. Plants with a visible inflorescence less than 2 months after treatment were not observed further

because it was assumed that they had initiated an inflorescence before treatments were applied. Excluding the plants that flowered naturally, 12 plants each remained in the control and uniconazole treatments and 13 plants in GA_3 treatment. At 93 days after treatment, six plants from each group were moved into a glasshouse and treated by pouring 10 ml of a 2% urea-water solution containing 10 mg ethephon into the plant center to induce flowering. The remaining plants were kept outdoors and data were collected on natural flowering.

Experiment 5.2 Crowns weighing approximately 175 g each were planted on February 17, 1993. The plants were grown in a glass-house for 266 days prior to treatment. The plants were maintained as described above. Plants were treated by pouring 10 ml of a water solution containing each growth regulator into the center of each plant to inhibit flowering on November 10, 1993. The treatments consisted of uniconazole (1.0 mg/plant), STS (19 mg/plant), AOA (1.0 mg/plant) and deionized water (control). The STS treatment was a mixture (a 1:4 molar ratio used for all the experiments) of silver nitrate and sodium thiosulfate. There were 6 plants each in the control and uniconazole treatments, and 5 plants each in the STS and AOA treatments. The plants were moved out of the glasshouse to the Magoon field area 13 days after the treatments were applied. For the AOA treatment, on January 12, 1994 (63 days after first treatment), a second application of 1.0 mg AOA per plant was made.

Experiment 5.3 Crowns weighing approximately 175 g each were planted on June 5, 1993 and the plants were grown outdoors. The plants were maintained as

described above. Plants were treated as described above on November 20, 1993 (168 days after planting) with uniconazole (1.0 mg/plant), paclobutrazol (10 mg/plant), STS (9.5 mg/plant), daminozide (20 mg/plant), and AOA (1.0 mg/plant). Deionized water was used as the control. A second application of STS or AOA was made on January 12, 1994 (53 days after the first treatment). There were 4 plants per treatment.

Experiment 5.4 Crowns weighing approximately 175 g were planted on January 7, 1994 and grown for 273 days in a glasshouse. The plants were maintained as described above. Plants were treated on October 7, 1994 (273 days after planting) with 0.5 or 2.5 mg uniconazole, 5 or 25 mg paclobutrazol, and 0.5 or 2.5 mg Fruitone by pouring 25 ml of a water solution containing the growth regulator into the center of each plant. Deionized water was used as the control. There were 8 plants in each of the 0.5 mg uniconazole (Uni-0.5), 5 mg paclobutrazol (Pac-5) and 0.5 mg Fruitone (Fru-0.5) treatments. There were 17 plants each in the control, 2.5 mg uniconazole (Uni-2.5), 25 mg paclobutrazol (Pac-25), and 2.5 mg Fruitone (Fru-2.5) treatments. For this latter group of treatments, 8 plants were used to observe the natural flowering response and 9 plants were sampled to measure ethylene production, ACCOase activity, and ACC and MACC contents in plant tissues. Sampling was done on 11/7/94 (31 days after treatment, DAT), 12/13/94 (67 DAT), and 1/20/95 (105 DAT) and 3 plants were sampled on each date. The 4th youngest emerged leaf was marked on each plant at the time of treatment, and this leaf (hereafter called the M-leaf) was used for the determination of ethylene production and ACCOase activity,

and leaf length was recorded to provide a measure of the effect of the growth regulator on leaf growth. Fourteen days after treatment (10/21/94), the plants were placed outdoors.

Experiment 5.5 Crowns weighing approximately 175 g were planted on April 4, 1994 and grown for 231 days out-of-doors. The plants were maintained as described above. The plants were moved into the glass-house and flushed with extra water to remove any fertilizer accumulated in the center of plants. The plants were first treated on December 2, 1994 (238 days after planting) with 1.0 mg aminoethoxyvinyl glycine (AVG), 1.0 mg uniconazole, or 10 mg STS by pouring 20 ml of a water solution containing each chemical into the center of each plant. Deionized water was used as the control. There were 13 plants in each treatment except 26 plants were treated with AVG (AVG-1). Half the plants treated with AVG were treated again on December 30, 1994 with 1.0 mg of the chemical (AVG-2). STS was applied 3 times to the 13 plants at three-week intervals. All other plants were treated with water when the second application of AVG and second and third applications of STS were made. Plants were placed out-of-doors on January 19, 1995. Three plants from each treatment were sampled on February 1, 1995 (61 days after treatment) to measure ethylene production by and ACCOase activity of leaf basal white and stem apical tissues.

Measurement of ethylene and ACCOase activity

The leaf basal white tissue was divided longitudinally into two parts and half was used for the measurement of ethylene and half for ACCOase activity. The

adjacent younger leaf was sampled for the measurement of ACC and MACC contents. The upper 1.0 cm of stem tissue was divided into four parts one quarter was used for each of the measurements ethylene, ACCOase activity, and ACC and MACC contents. About 1.0 g of tissue was put into a 17.5 ml test tube, sealed with a serum stopper and incubated for about 2 hours (exceptions will be noted) at room temperature (about 25 °C) and in room light. After incubation, a 1.0 ml gas sample was withdrawn from the headspace and injected into a gas chromatograph (GC) equipped with a flame ionization detector (Min and Bartholomew, 1993; see Appendix).

ACCOase activity was estimated by measuring the conversion of ACC to ethylene in vitro (Starrett and Laties, 1990; Min and Bartholomew, 1993; see Appendix). About 1.0 g of fresh tissue was dipped in a 1.0 mM ACC solution for 2 minutes, blotted dry, placed in a 17.5 ml test tube, sealed with a serum stopper, and incubated for approximately 2 hours, after which ethylene was measured as described above.

Determination of ACC and MACC

The procedures of Lizada and Yang (1979) and Sitrit et al. (1988) were followed to determine the contents of ACC and MACC. About 1.0 g of fresh tissue was weighed, frozen with liquid nitrogen and stored at -20 °C. At the time of measurement, each sample was macerated in a mortar with 20 mg polyvinlypyrolidine (PVPP), a small amount of sand, and 6 ml of 80% ethanol. After centrifuging at 6000 g, the supernatant was transferred to another tube. The pellet was re-suspended

with 2 ml 80% ethanol and centrifuged again. The supernatant was combined and dried in vacuum at room temperature. The dry residue was dissolved in 2 ml of water and 2 ml of chloroform. The tube was shaken and centrifuged. The upper aqueous phase was pipetted off and subdivided into three 0.6 ml samples. One fraction was used for the determination of ACC by adding 1.0 ml of 8 mM $HgCl_2$ and 0.15 ml of a cold mixture of 5.25% NaOCl (commercial bleach) and saturated NaOH (2:1, v/v). The conversion efficiency of ACC to ethylene in each sample was determined by adding 2 nmol of pure ACC in 25 μl water to a replicate sample prior to adding $HgCl_2$. One ml of 6 M HCl was added to one replicate sample and incubated for 3 hour at 100 °C to hydrolyze MACC to ACC. After incubation 1.0 ml of 6 M NaOH was added to neutralize the solution, and the ACC content was determined. MACC content was calculated by subtraction of the ACC content measured in non-hydrolyzed samples.

5.3 Results

5.3.1 Effects of growth regulators on vegetative growth of pineapple

Uniconazole and paclobutrazol significantly retarded leaf elongation relative to the control (Table 5.1). The extent of the inhibition increased as the amount (concentration) of growth regulator was increased. The higher concentration of Fruitone also significantly inhibited leaf elongation, but there was no significant difference between the control and the low concentration of Fruitone. The Fru-2.5 treatment killed the basal tissue of some leaves where it was concentrated in the whorl

of younger leaves, and caused twisting of younger leaves. The stem apical meristems of 6 plants among 17 treated were killed. Fru-0.5 caused a slight twisting of the young leaves but there were no other visible signs of injury.

Table 5.1. Effects of Fruitone (Fru), paclobutrazol (Pac) and uniconazole (Uni) on leaf length and leaf appearance rate.

Treatment	Leaf length† (cm)	Leaf appearance rate‡ (leaf/day) 1	2
Control	78.6	0.232	0.216
Fru-0.5§	74.6	0.252	0.236
Fru-2.5	58.5	0.250	0.210
Pac-5	55.5	0.170	0.308
Pac-25	40.5	0.143	0.266
Uni-0.5	62.5	0.221	0.268
Uni-2.5	34.8	0.195	0.313
LSD (0.05)	6.9	0.024	0.024

† The length of the longest emerged leaf after application of the growth regulators. Leaf length was measured on March 9, 1995, 153 days after application of the growth regulators (n=8).
‡ Column 1 is the rate of leaf appearance between October 7, 1994 (the day plants were treated) and December 7, 1994; column 2 is the leaf appearance rate between December 8, 1994 and February 8, 1995 (n=8).
§ Treatments were in active ingredient, Fru, 0.5 and 2.5 mg plant^{-1}; Pac, 5.0 and 25.0 mg plant^{-1}; and Uni, 0.5 and 2.5 mg plant^{-1}.

GA_3, which was used in Experiment 5.1, promoted leaf elongation, but no data on length were collected. The 19 mg per plant STS treatment in Experiment 5.2 caused tip burn of some young leaves, but there was no damage in other experiments where lower concentrations were used. Other chemicals including AOA, AVG, and daminozide had no visual effect on vegetative growth and caused no obvious injury of pineapple.

Both uniconazole and paclobutrazol decreased the rate of leaf appearance in the first two months after treatment (Table 5.1). In the second two month period, the rate of appearance was significantly greater in those treatments than in the control (Table 5.1). This result suggests that the lower leaf appearance rate in the first two months likely was due to the inhibition of leaf elongation rather than to inhibition of the leaf initiation rate. Fruitone had no effect on leaf appearance.

The effects of uniconazole and paclobutrazol on leaf area and dry matter accumulation in different parts of the plant were measured when plants were cut for measurement of ethylene (Table 5.2). The effect of Fruitone on leaf area was not determined because of the injury to some of the plants. Both uniconazole and paclobutrazol significantly inhibited leaf area, and fresh and dry weight of the young leaves, including the 4th youngest leaf marked at the time of treatment. The leaf area, and fresh and dry weight of leaves older than the marked leaf were less affected by the growth regulators. Although stem fresh weight in the paclobutrazol treatment was significantly higher than that in control and uniconazole treatments, stem dry weights among treatments were not significantly different. These results indicate stem dry matter accumulation was not or was less influenced by uniconazole and paclobutrazol treatments than leaf growth.

Table 5.2. Effects of 25 mg paclobutrazol (Pac-25) and 2.5 mg uniconazole (Uni-2.5) per plant on leaf area and dry matter partitioning of pineapple†.

	Treatment			
	Control	Pac-25	Uni-2.5	LSD (0.05)
Young-leaf area (cm^2)‡	5011.8	2091.9	2861.1	849.3
Other leaf area (cm^2)	12143.7	13050.0	10305.1	ns
Total leaf area (cm^2)	17155.5	15141.6	13167.3	2476.5
Young-leaf FW (g)	863.5	409.7	539.3	149.3
Other leaf FW (g)	1790.5	1947.1	1429.0	ns
Total leaf FW (g)	2654.0	2356.9	1968.3	432.4
M-leaf DW (g)	120.3	65.2	46.9	20.7
Other leaf DW (g)	279.6	286.1	214.1	ns
Dead Leaf DW (g)	33.2	25.9	32.2	ns
Total leaf DW (g)	433.0	358.9	311.4	75.0
Stem FW (g)	382.8	505.5	394.1	101.2
Stem DW (g)	101.4	127.7	105.9	ns

† Plants (n=3) were harvested on January 20, 1995 (105 days after treatment).
‡ Area of green leaves expanded after treatment, which was identified by marking the 4th youngest leaf (M-leaf) at the time of treatment. "Other leaf" refers to leaves older than the marked leaf.
ns is not significant at the 0.05 level of probability by F-test.

5.3.2 Effects of growth regulators on ethylene production by, ACCOase activity of, and ACC and MACC contents of plant tissues

Because some plants in the Fru-2.5 treatment were damaged, the plants were sampled only once to determine the treatment effect on ethylene metabolism (Table 5.3). Compared with the control, Fruitone increased ethylene production by stem tissue four-fold, stem tissue ACC oxidase activity 2.5-fold, and MACC content in stem tissue 44-fold, while the ACC content was not significantly different from the control. These results indicate that Fruitone, a synthetic auxin, stimulated ethylene production mainly by increasing ACC synthase. The content of ACC was not

significantly changed because the higher ACC oxidase activity promoted ACC oxidation to ethylene. Further, the conjugation of ACC to form MACC, which provides a mechanism to maintain a relative constant level of ACC, suggests a rate of ACC production much greater than could be oxidized to ethylene.

In Experiment 5.4, ethylene production was assayed three times over a period of about three months. In all three samplings, ethylene production by stem tissue was not significantly affected by uniconazole or paclobutrazol (Table 5.3). Rates of ethylene production by stem tissue were comparable at all three measurement dates. ACCOase activity in stem tissue was increased significantly in plants treated with paclobutrazol in the first and second samplings and with uniconazole in the second sampling. By the January, 1995 sampling, all three control plants had floral primordia that were 1 to 2 weeks old and the ACCOase activity in the stem tissue of the control was significantly higher than that in stem tissues of the paclobutrazol and uniconazole treatments. The contents of ACC and MACC in stem tissue were not influenced by paclobutrazol and uniconazole treatments.

In December, 1994, the 4th youngest emerged leaf (M-L) marked at the time of treatment was sampled. Ethylene production and ACCOase activity of the M-L basal tissue of plants treated with paclobutrazol and uniconazole were significantly less than the control (Table 5.3). The ACC and MACC contents of the leaf basal tissue were not determined. In January, 1995, ethylene production by the M-leaf was only a small fraction of that measured in December, 1994, and there was no difference in ethylene production and ACCOase activity of the M-leaf basal tissue

among treatments. This result indicates there was some developmental regulation of ethylene production and ACCOase activity (Min and Bartholomew, 1993; see Appendix). The leaf 6 younger (M6-L) leaf than the M-leaf had much higher ethylene production (statistically not significant because of large variation) and significantly higher ACCOase activity in the control than those in the paclobutrazol and uniconazole treatments. The contents of ACC and MACC in basal tissue of the leaf just younger than the M-L (M1-L) and stem apical tissues were not significantly affected by the treatments.

In Experiment 5.5 where plants were treated with AVG, STS, and uniconazole, some plants in all treatments had initiated inflorescences at the time of sampling (February 1, 1995, 62 days after treatment). There were no significant effects of AVG, uniconazole, or STS on ethylene production or ACCOase activity of the marked leaf basal or stem tissues. However, when comparing reproductive and vegetative plants, both leaf and stem ACCOase activities were significantly ($P=0.05$ or 0.01) higher in the reproductive than in vegetative plants (Table 5.4). Ethylene production by leaf tissue in reproductive plants was 4 times higher than that in vegetative plants, but the difference was not significant due to a large variance (Table 5.4). There was no difference in ethylene production by stem tissues. It is known that exogenously applied ethylene induces pineapple flowering (Bartholomew and Criley, 1982). These results indicate that increased ethylene production in leaf tissues, due at least in part to the increase in ACCOase activity, may be one of the factors involved in natural induction of flower development in pineapple.

Table 5.3. Effects of 25 mg paclobutrazol (Pac-25), 2.5 mg uniconazole (Uni-2.5) and 2.5 mg Fruitone (Fru-2.5) per plant on ethylene production by, and 1-aminocyclopropane-carboxylic acid (ACC) oxidase (ACCOase) activity, ACC and malonyl-ACC (MACC) contents of plant tissue.

	Treatment				
	Control	Pac-25	Uni-2.5	Fru-2.5	LSD (0.05)
Nov. 7, 1994 (31 DAT)†					
S-ethylene (nl $gFW^{-1}h^{-1}$)‡	1.84	2.47	1.76	7.72	0.93
S-ACCOase (nl $gFW^{-1}h^{-1}$)	24.92	49.94	30.49	62.61	19.97
S-ACC (nmol gFW^{-1})	2.17	3.45	5.10	4.61	ns
S-MACC (nmol gFW^{-1})	1.21	1.26	ND	53.60	13.89
Dec. 13, 1994 (67 DAT)					
S-ethylene (nl $gFW^{-1}h^{-1}$)	3.07	6.42	8.26	-	ns
S-ACCOase (nl $gFW^{-1}h^{-1}$)	29.64	86.26	76.98	-	20.84
S-ACC (nmol gFW^{-1})	0.90	1.00	1.00	-	ns
S-MACC (nmol gFW^{-1})	3.20	1.90	1.20	-	ns
M-L-ethylene (nl $gFW^{-1}h^{-1}$)	10.21	2.98	2.88	-	2.45
M-L-ACCOase (nl $gFW^{-1}h^{-1}$)	71.71	12.39	8.72	-	29.35
M-L length (cm)	70.3	47.8	52.6	-	11.3
Jan. 20, 1995 (105 DAT)§					
S-ethylene (nl $gFW^{-1}h^{-1}$)	3.61	2.18	2.99	-	ns
S-ACCOase (nl $gFW^{-1}h^{-1}$)	72.14	42.76	50.26	-	21.51
S-ACC (nmol gFW^{-1})	1.08	0.89	1.61	-	ns
S-MACC (nmol gFW^{-1})	2.41	4.61	6.07	-	ns
M-L-ethylene (nl $gFW^{-1}h^{-1}$)	0.50	0.58	0.51	-	ns
M-L-ACCOase (nl $gFW^{-1}h^{-1}$)	1.56	1.70	2.00	-	ns
M1-L-ACC (nmol gFW^{-1})	0.38	1.09	1.19	-	ns
M1-L-MACC (nmol gFW^{-1})	0.73	1.92	1.93	-	ns
M6-L-ethylene (nl $gFW^{-1}h^{-1}$)	3.13	0.69	0.48	-	ns
M6-L-ACCOase (nl $gFW^{-1}h^{-1}$)	38.20	2.27	1.55	-	31.45
M-L length (cm)	75.1	49.7	51.4	-	5.0
M6-L length (cm)	69.7	24.5	29.7	-	6.9

† Plants were sampled 31, 67 and 105 days after treatment (DAT), respectively. The incubation times were 2.25, 3.75, and 4 hours, respectively.
ns, not significant at the 0.05 leave of probability by F-test; ND, not detectable; -, not measured.
‡ S, stem apical tissue; L, leaf basal white tissue; M-L, the 4th youngest leaf marked at the time of treatment; M6-L, the 6th younger leaf than the M-leaf; M1-L, the first younger leaf than the M-leaf.
§ The control plants had young inflorescences that were estimated to be 1 to 2 weeks old at the time of sampling; all paclobutrazol and uniconazole treated plants were vegetative.

Table 5.4. Ethylene production and ACCOase activity of plant tissue for vegetative and reproductive pineapple plants.

	Reproductive plants	Vegetative plants
	- - - - - - - - - nl $g^{-1}h^{-1}$ - - - - - - - - -	
Leaf-ethylene	21.34†	5.06
Stem-ethylene	2.96	2.94
Leaf-ACCOase	131.15	52.88*
Stem-ACCOase	68.51	43.55**

† Data are means of 9 reproductive plants and 6 vegetative plants.
*, ** significant at 0.05, 0.01 level of probability by t-test.

5.3.3 Effects of growth regulators on pineapple flowering

The primary results of Experiment 5.1 were reported (Min and Bartholomew, 1994). All control and GA_3 treated plants were induced to flower with ethephon while only 3 of 6 plants treated with 10 mg of uniconazole were induced to flower. The 3 unforced plants were treated with a second application of 10 mg ethephon 73 days after the first application. The 3 plants still remained vegetative but each plant produced 22 shoots (suckers), while no visible shoots were produced on plants only treated with uniconazole. There was no significant difference in fruitlet number per inflorescence between control (112) and GA_3 treatments (119), but uniconazole treated plants had significantly fewer fruitlets (69) per inflorescence.

For plants used to observe the natural flowering responses, all control and GA_3- treated plants flowered naturally. Their inflorescences emerged about 5 months after the treatments were applied. None of the 6 plants treated with uniconazole flowered at that time and 3 of 6 plants flowered 19 months (March, 1994) after

treatment, 2 plants flowered about 30 months after treatment, and 1 plant was still vegetative by April, 1995 (32 months after treatment).

Uniconazole significantly delayed pineapple inflorescence appearance compared with the control (Experiment 5.2; Table 5.5), while STS, and AOA treatments were not significantly different from the control. Because the plants flowered at different times, which resulted in differences in plant size at the time of flowering, and developed in different environments, no attempt was made to compare the effects of treatments on fruit development. There were no visual differences in the fruit size or morphology and the fruitlet numbers were not significantly different (Table 5.5).

In Experiment 5.3, both uniconazole and paclobutrazol delayed pineapple inflorescence appearance for more than 6 months, while STS, daminozide and AOA had no significant effect (Table 5.5). There was no significant difference in fruitlet number per fruit among the treatments though flowering time was different. The size of fruits was not determined. Comparing the effects of uniconazole (1.0 mg per plant in both experiments) in Experiment 5.2 where the inflorescence appearance was delayed 76 days and Experiment 5.3 where the inflorescence appearance was delayed 206 days, it may be that the age or size of plants at the time of treatment influenced the effectiveness of the growth regulator. The plant age at the time of treatment in Experiment 5.2 was 266 days while in Experiment 5.3 plants were 168 days old.

In Experiment 5.4, all plants in the control flowered while flower induction of Fru-0.5 treated plants was delayed by an average of 38 days (Table 5.6). As of May 20, 1995, 6 plants in the Pac-5 and 5 plants in the Uni-0.5 initiated inflorescence

development but later than the control. However, all plants in Pac-25, Fru-2.5, and Uni-2.5 remained vegetative.

Both AVG (AVG-1, AVG-2) and STS did not delay pineapple natural flowering (Experiment 5.5) (Table 5.6). Inflorescences of three out of 10 plants in uniconazole treatment appeared about 3 months after treatment. The average time of inflorescence appearance in the uniconazole treatment suggested these plants initiated floral primordia prior to or shortly after application of uniconazole.

In summary, the two growth retardants uniconazole and paclobutrazol, and Fruitone, a synthetic auxin, delayed or inhibited pineapple flowering.

Table 5.5. Effects of aminooxyacetic acid (AOA), daminozide, paclobutrazol, silver thiosulfate and uniconazole on the inflorescence appearance time and fruitlet number per fruit of naturally induced pineapple.

Treatment	DIA†	Fruitlet Number
Exp. 5.2‡		
Control	141±34	128±18
AOA	128±18	142±8
Uniconazole	219±26	133±15
Silver thiosulfate	170±61	135±17
LSD (0.05)	48	ns
Exp. 5.3§		
Control	181±11	128±4
AOA	139±22	125±7
Daminozide	192±17	129±7
Paclobutrazol	368±44	116±3
Uniconazole	387±40	116±7
Silver thiosulfate	228±12	131±8
LSD (0.05)	167	ns

† DIA, average days from application of growth regulators to inflorescence appearance.
‡ In Experiment 5.2, plants were treated on November 10, 1993, 266 days after planting. There were 6 plants each in the control and uniconazole (1.0 mg plant^{-1}) treatment and 5 plants each in the AOA (0.1 mg plant^{-1}) and silver thiosulfate (STS) (19 mg plant^{-1}) treatments.
§ In Experiment 5.3, plants were treated on November 20, 1993, 168 days after planting (n=4).
ns, not significant at the 0.05 level of probability by F-test.

Table 5.6. Effects of aminoethoxyvinylglycine (AVG), Fruitone, paclobutrazol, silver thiosulfate and uniconazole on the natural flowering of pineapple.

Treatment	Total plants	Reproductive plants†	DIA‡
Exp. 5.4§			
Control	8	8	137
Fruitone-0.5	8	8	175
Fruitone-2.5	8	0	
Paclobutrazol-5	8	6	164
Paclobutrazol-25	8	0	
Uniconazole-0.5	8	6	158
Uniconazole-2.5	8	0	
Exp. 5.5¶			
Control	10	9	102
AVG-1	10	8	105
AVG-2	10	10	105
Silver thiosulfate	10	9	118
Uniconazole	10	3	93

† Plants with a visible inflorescence by the end of April, 1995.
‡ DIA, average days from application of growth regulators to inflorescence appearance. Values are means for the reproductive plants.
§ The plants were treated on October 7, 1994 (273 days after planting); 0.5 and 2.5 mg Fruitone, 5.0 and 25.0 mg paclobutrazol, and 0.5 and 2.5 mg uniconazole per plant were applied.
¶ The plants were treated on December 2, 1994 (238 days after planting). One mg of AVG per plant was applied once (AVG-1) and a second 1.0 mg application was made 28 days after the first one (AVG-2); 10 mg silver thiosulfate per plants were applied three times at three-week intervals; and 1.0 mg uniconazole per plant was applied.

5.4 Discussion

Flower induction of pineapple depends on the size and physiological status of the plants and on daylength and temperature (Bartholomew and Malézieux, 1994). Based on the finding that flowering is induced by exogenously applied ethylene, ethephon, or auxins that stimulate ethylene production, it is hypothesized that natural flowering of pineapple is induced by endogenously produced ethylene or by changes in susceptibility to it, or both (Min and Bartholomew, 1993; see Appendix). In this study (Experiment 5.5), plants that had initiated an inflorescence had significantly greater ACCOase activity in leaf and stem tissues than did vegetative plants, and ethylene production by leaf tissue was also greater. The only way to unequivocally prove the above hypotheses would be to inhibit endogenously produced ethylene by genetically engineering plants as was done with the ACCOase or ACC synthase mRNA anti-sense transgenic tomato plants (Hamilton et al, 1990; Klee and Romano, 1994) or to find a mutant plant deficient in ethylene perception as the *Never Ripe* tomato mutant (Lanahan et al., 1994; Bowler and Chua, 1994).

In this study, STS, AOA and AVG did not delay or inhibit pineapple flowering. Ethylene production was inhibited when plant tissue was dipped in AOA solution (Min and Bartholomew, 1993; see Appendix) and STS or silver nitrate applied prior to ethephon treatment blocked flower induction by ethephon (Sanford and Bartholomew, 1981; Min and Bartholomew, 1993; see Appendix) and reduced natural flowering (Millar-Watt, 1981). The failure of STS to block natural flowering of pineapple in this study may be due to biological dilution or an inadequate amount

of STS applied which did not reach the threshold required to block ethylene action (concentrations greater than 1 g L^{-1} are phytotoxic). The failure of AOA and AVG could also be due to biological dilution or degradation. And there was no any published information regarding the uptake of these compounds in plants.

Uniconazole and paclobutrazol inhibit extension growth in a wide range of species (Davis et al., 1988; Davis and Curry, 1991). In this study, both chemicals inhibited leaf elongation of pineapple and also delayed or inhibited pineapple natural flowering. Their known mechanism of action is that they inhibit gibberellin(s) biosynthesis. For example, uniconazole inhibited gibberellin content in *Lycopersicon esculentum* (Yamaji et al., 1991). Paclobutrazol also inhibited water stress-induced ethylene and polyamine biosynthesis (Wang and Steffens, 1985) and ABA accumulation in apple seedling leaves (Wang et al., 1987) and wheat seedlings (Buta and Spaulding, 1991). Uniconazole-P, an active optical isomer of uniconazole, did not change IAA and ABA levels but stimulated ethylene production (1.8 times the control) and cytokinin content (3.4 times in *trans*-ribosyl zeatin and 3 times *trans*-zeatin) relative to the control in rice shoots (Izumi et al., 1988). These triazol growth regulators also interfered with sterol metabolism (Lurssen, 1987; Grossmann, 1990). The triazol compounds also inhibited ethylene production by barley and oilseed rape (Grossmann et al., 1989), wheat and soybean (Krause et al., 1991), mung bean (Hofstra et al., 1989) and apple seedling (Wang and Steffens, 1985).

In this study, both uniconazole and paclobutrazol inhibited leaf ethylene production and decreased ACCOase activity in pineapple . The decrease in ethylene

production by leaf tissue could explain in part the delay or inhibition of pineapple flowering, although the involvement of other hormones such as a decrease in gibberellin(s) quantity or activity and altered plant susceptibility to ethylene can not be excluded.

The inhibiting effect of Fruitone on pineapple ratoon plant flowering reported by Scott (1993) was confirmed. However, Scott (1993) attributed the inhibition to reduced vegetative growth rather than directly interfering with the induction process. Analysis of ethylene production and ACC and MACC contents in the stem tissue of Fruitone-treated plants showed that Fruitone produced an auxin-like response in stimulating ethylene production. Fruitone at low concentrations induced pineapple to flower (Gowing and Leeper, 1960), while higher concentrations of NAA, another synthetic auxin, inhibited flowering (Gowing, 1956; Millar-Watt, 1981). Therefore, the inhibiting effect of Fruitone on flowering may be due at least in part to its auxin activity. The reason for the inhibiting effects of auxin on flowering at higher concentrations is not known. The flowering of pineapple induced by geotropic stimulation (Van Overbeek and Cruzado, 1948b) suggested the involvement of auxin(s) in the control of this process. The complex interactions of auxin(s) and ethylene, and other plant hormones, as well as environmental factors make the flowering process, in part or in a whole, a mystery in this plant as well as other flowering plants (Abeles et al., 1992; Mattoo and Suttle, 1991; Bernier, 1988; McDaniel, 1994; Bernier et al., 1993; Kinet, 1993).

The finding that uniconazole, paclobutrazol and Fruitone inhibited pineapple

flowering may warrant further field trials to test their application potential. These compounds may also provide a tool to further probe the physiology of this process, which may improve our understanding of the underlying mechanism(s) and create new technology in the management of pineapple.

CHAPTER 6

SUMMARY AND CONCLUSIONS

In the research described here, the effects of temperature, water, and growth regulators on pineapple natural flowering or ethephon-induced flowering were examined. The effects of these factors on the biosynthesis of ethylene, which is hypothesized to be the signal that initiates inflorescence development of pineapple, leaf TA, and floral initiation and development were determined.

Plants grown for about 7 months outdoors then exposed to a day/night temperature of 30/30 °C for 30 and 51 days produced significantly less ethylene with lower 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACCOase) activity of stem and leaf basal white tissues than did plants grown at 30/20 °C in one trial but not in the other. The flowering induction response to ethephon was consistent for both trials. There were some plants unforced by ethephon in the 30/20 °C pre-forcing - 30/30 °C post-forcing treatment in both trials. The fruitlets per fruit was about 100 for plants grown continuously at 30/20 °C in both trials, and 14 in one experiment and 37 in a second experiment for plants grown continuously at 30/30 °C. The fruitlet number was about 80 in the 30/30 °C pre-forcing - 30/20 °C post-forcing treatment. Leaf am TA of plants grown at 30/30 °C was about 61% of that of plants grown at 30/20 °C. Low ethylene production, ACCOase activity, and reduced CO_2 dark fixation may account in part for the reduced flower induction response and lower

fruit weight in environments having warm night temperatures.

Ethylene production and ACCOase activity in leaf tissue increased while stem tissue values were unchanged by water excess stress in one trial. In a completely waterlogged trial, ethylene production by leaf and stem tissues and ACCOase activity in leaf tissue of stressed plants were not different from the control, whereas ACCOase activity in stem tissue was decreased by water excess stress. Ethylene production by leaf basal tissue and ACCOase activity in both leaf basal and stem tissue decreased significantly in response to water deficit stress, but ethylene production by green leaf and stem tissues and ACCOase activity in green leaf tissue were not different from the control. Both water excess and water deficit stress significantly reduced leaf am TA, the result of CO_2 dark fixation. Neither water excess nor water deficit stress induced natural flowering of pineapple, showing that water is not a major factor affecting the natural flowering of pineapple. Water deficit stress decreased plant susceptibility to ethephon, perhaps due to the rapid onset and extreme level of stress that occurred in these potted plants.

Both waterlogging and water deficit significantly reduced leaf RWC and leaf am TA. After relief of the treatments by watering or draining excess water from waterlogged plants, leaf RWC and am TA returned to normal more quickly in water deficit than in waterlogging treatments. Fruitlet numbers were decreased more by water deficit than by waterlogging, while waterlogging reduced fruit size more than did water deficit stress.

Among the chemicals tested for their potential to delay or inhibit pineapple

flowering, uniconazole, paclobutrazol and Fruitone delayed or inhibited pineapple flowering, while AOA, AVG, STS, and daminozide did not. Fruitone, a synthetic auxin, stimulated ethylene production, but the mechanism by which it delayed flowering is not known. Uniconazole and paclobutrazol, growth retardants that inhibit gibberellin biosynthesis, inhibited leaf elongation and decreased leaf area. The activity of uniconazole in the inhibition of vegetative growth and flowering was approximately ten times stronger than paclobutrazol. Uniconazole and paclobutrazol significantly inhibited ethylene production by and ACCOase activity of leaf tissue. Decreased ethylene production by leaf tissue could be one factor responsible for delayed flowering in treated plants. Based on the results of this research, further field evaluation of the effectiveness of uniconazole and paclobutrazol in delaying or inhibiting natural flowering of pineapple seems warranted.

APPENDIX

EFFECTS OF GROWTH REGULATORS ON ETHYLENE PRODUCTION AND FLORAL INITIATION OF PINEAPPLE

Xiangjia Min and Duane P. Bartholomew

Abstract

Natural flowering of pineapple (*Ananas comosus* (L.) Merrill) can reduce yields and disrupt crop scheduling. In an effort to better understand the mechanism(s) of flowering, pineapple tissue ethylene production and the effects of growth regulators on it were investigated. Ethylene production (fresh weight basis) by basal white tissue of the youngest physiologically mature (D) leaf (basal tissue) ranged from 1.97 to 3.99 nl g^{-1} h^{-1}; that produced by stem apical (stem) tissue ranged from 0.52 to 1.37 nl g^{-1} h^{-1}. Ethylene was not detected from green leaf tissue. Ethylene production by basal tissue was correlated ($r = 0.74$) with ethylene-forming enzyme (EFE) activity of that tissue but not with 1-aminocyclopropane-1-carboxylic acid (ACC) levels. Ethylene production by stem tissue was more highly correlated with ACC level ($r = 0.88$) than with EFE activity ($r = 0.21$). Ethylene production by stem tissue and basal tissue were positively correlated ($r = 0.64$). Ethylene production by basal tissue was somewhat correlated with plant size ($r = 0.44$) while that of the stem was not. EFE activity in leaf and stem tissue were not well related to plant size. Ethylene production by stem tissue of ethephon treated plants was at least two-fold greater than the controls up to five days after treatment. Stem tissue of plants treated

with naphthaleneacetic acid (NAA) produced up to ten times more ethylene than did controls. All plants treated with ethephon or NAA were induced to flower. Ethylene production by stem tissue dipped in a solution containing 2.0 g l^{-1} gibberellic acid (GA_3) was significantly lower than controls, but ethylene production by stem tissue of GA_3 treated plants was not different from controls. Ethylene production by stem tissue dipped in a solution containing amino-oxyacetic acid was significantly inhibited. Dikegulac sodium, paclobutrazol and uniconazole had no effect on ethylene production or EFE activity of excised tissue dipped in solutions, or on treated intact plants. GA_3, dikegulac sodium, paclobutrazol, and uniconazole applied prior to forcing did not block ethephon induced flowering. GA_3 promoted peduncle elongation, but decreased inflorescence weight while plants treated with paclobutrazol and uniconazole had a shortened peduncle, but inflorescence weights that were comparable to the control. Plants treated with silver thiosulfate (STS) followed by ethephon a week later were not forced. The data indicate that ethylene plays a primary role in floral initiation and that GA_3 may be involved in inflorescence development.

Additional key words: naphthaleneacetic acid, gibberellic acid, ethylene-forming enzyme, *Ananas comosus*, growth retardants

1. Introduction

In commercial practice, pineapple is induced to flower (forced) with acetylene, ethylene, ethephon, and auxins such as naphthaleneacetic acid (NAA) (Bartholomew and Criley, 1984). Acetylene and ethylene are unsaturated gases that have similar chemical structures while ethephon readily degrades to ethylene. NAA stimulates the plant to produce ethylene and Burg and Burg (1966) speculated that ethylene, not auxin, caused pineapples to flower.

Ethylene is an endogenous plant hormone that regulates plant growth and development. Biochemically, ethylene production is controlled by the substrate concentration of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, and the activity of ethylene forming enzyme (EFE) (Yang and Hoffman, 1984; Kende, 1993). ACC synthase is the primary factor limiting ACC synthesis and ethylene production (Yang and Hoffman, 1984). However, there is increasing evidence that in some plants the activity of EFE also changes in response to factors that influence ethylene production (Kende, 1993).

The role ethylene plays in natural flowering of pineapple and other bromeliads (de Greef et al., 1983) is not known. Burg and Burg (1966) could not detect ethylene from intact 8 month-old potted pineapple plants. De Greef et al. (1983) reported that flowering maturity of *Aechmea victoriana* was correlated with the plants capacity to convert applied ACC to ethylene, but no studies on the relationship between ethylene production and natural flowering were found.

There is a need to understand the flowering physiology of pineapple in order

to improve the effectiveness of forcing and to inhibit natural flower induction, which usually occurs in the December-January period in Hawaii. We hypothesize that natural flowering of pineapple is induced by ethylene produced by the plant or by increased plant sensitivity (susceptibility) to ethylene or both and practices that inhibit ethylene production or action or decrease plant sensitivity to ethylene, or both, can inhibit precocious flowering in pineapple. The objectives of the studies reported here were: a) to characterize the relationship between the ethylene production, its precursor ACC, and EFE activity in pineapple tissue, and their relation to plant size, and b) to determine the effects of certain growth regulators that have been reported to inhibit ethylene production or action in other plants on ethylene production and floral initiation in pineapple.

2. Material and Methods

Experiments were carried out using field-grown Smooth Cayenne plants on the Del Monte pineapple plantation, Kunia, Hawaii or using pot-grown Smooth Cayenne plants kept in a glasshouse or growth chamber. Ethylene production was assayed by placing about 1 g of fresh tissue from the D-leaf white basal (hereafter referred to as basal tissue), white-green, or green tissue or stem apical (stem) tissue into a 17.5 ml test tube, sealing the tube with a serum stopper, and incubating the sample at 25°C in the dark or in room light for 2 h. Exceptions to the above procedure will be noted. After incubation, a 1.0 ml gas sample was withdrawn from the headspace and injected into a gas chromatograph (GC) equipped with a flame ionization detector for determination of the ethylene concentration.

For the measurement of EFE activity, about 1 g of fresh tissue was dipped for 30 s in a 1.0 mM ACC-water solution, blotted dry, placed in a 17.5 ml test tube and incubated as described above after which ethylene was measured by GC. ACC was extracted from about 1.0 g of fresh tissue following the procedure of Sitrit et al. (1988) and quantified by oxidizing it to ethylene following the procedure of Lizada and Yang (1979).

To examine the effects of ethephon, NAA, and GA_3 on ethylene production and forcing of field-grown pineapple, plants weighing about 1.5 kg fresh weight were selected. Plants were treated by pouring 10 ml of a 2% urea:water solution (w:w) containing the growth regulators into the plant center. Treatments consisted of 10 or 20 mg of ethephon, 0.5 or 1.0 mg of NAA, or 50 mg of GA_3. Ethylene production by stem tissue was measured daily for the first 5 days and then again on day 8 and 10 after treatment. Forcing percentage was determined by dissection 3 weeks after treatment.

The effects of GA_3, uniconazole, dikegulac sodium, and AOA on stem tissue ethylene production were assayed by dividing single stem tips (about 0.5 cm thick) into 4 parts weighing approximately 0.5 g each. The tissue was dipped for 5 minutes in deionized water solutions containing either 2.0 g l^{-1} of GA_3, 1.0 g l^{-1} of uniconazole, 1.0 g l^{-1} dikegulac sodium, or 0.1 g l^{-1} of AOA. After dipping, the tissue was blotted dry and transferred to a test tube and ethylene production was measured as described above.

The effects of GA_3, dikegulac sodium, and paclobutrazol on ethylene

production by stem tissue of intact plants was also investigated. Four plants were treated with 10 ml of a solution containing either 20 mg GA_3, 10 mg dikegulac sodium, or 20 mg paclobutrazol (20 mg/plant). Chemicals were applied in the plant center and stem tissue was collected for EFE and ethylene production assays 24 hours after treatment.

To assess the effects of GA_3 and daminozide applied prior to ethephon forcing on floral initiation and development, 6-month old pot-grown plants were treated by pouring 10 ml of a water solution containing the growth regulators into the plant center. Groups of 10 plants were treated with either 20 mg GA_3, 20 mg daminozide or water. Eight days after treatment, all plants were forced by pouring 5 ml of a 2% urea solution containing 5 mg ethephon into the plant center. Six weeks after forcing, floral initiation was evaluated by dissection. Where forcing had occurred, the weight of the developing inflorescence was recorded.

A field experiment was installed to further test the ability of several chemicals to inhibit ethephon forcing of pineapple. Each treatment was applied to 30 plants with three replications. Individual plants weighing about 2.0 kg fresh weight were pre-treated with 10 ml of water containing 20 mg of daminozide, mepiquat chloride, paclobutrazole, GA_3 or silver thiosulfate (STS), or 10 mg of dikegulac-sodium or uniconazole into the plant heart. Distilled water was used as the control. STS was a mixture (1:4 molar ratio) of silver nitrate and sodium thiosulfate. One week after applying these chemicals, 10 mg of ethephon in 10 ml of a 2% urea solution were applied to test for susceptibility to forcing. Three weeks after forcing, apical

meristems of 15 plants per treatment were exposed by dissection and the percentage of plants showing a young inflorescence was recorded. The flowering status of the remaining plants was determined three months after forcing. At six months after forcing, peduncle length was measured, eyes per fruit were estimated and the number of propagules (suckers, hapas, slips) were counted.

3. Results

3.1. *Ethylene production, EFE activity and ACC level in pineapple tissues*

Leaf basal tissue produced more ethylene than the more distal white-green tissue; ethylene production by green leaf tissue was not detectable (Figure 1). Stem tissue produced significantly less ethylene than did basal tissue. The basal tissue and stem tissue were used in all later studies because of the high rate of ethylene production by basal tissue and because the stem is where physiological changes associated with flower initiation occur.

Ethylene produced by basal tissue was well correlated with EFE activity ($r=0.74$, significant at $p > 0.01$) in that tissue (Figure 2a); ethylene produced by stem tissue was poorly correlated with EFE activity (Figure 2b). Stem ethylene production was positively correlated with ACC content in that tissue, but basal tissue ethylene production was poorly correlated with ACC level (Figure 3a and 3b). However, the small sample size makes the results somewhat inconclusive.

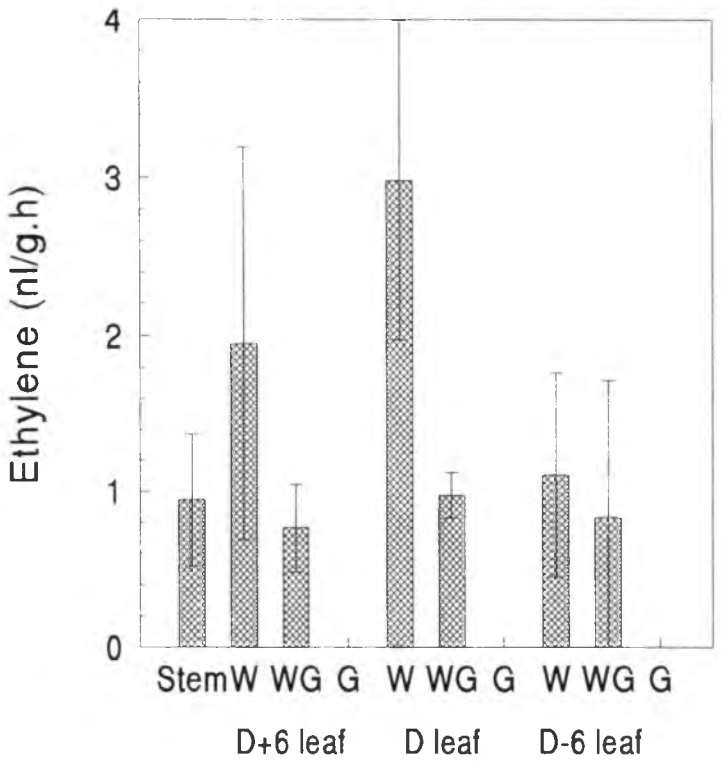

Figure 1. Ethylene production in different tissue of pineapple. The number is the mean from 5 plants. The tissue was incubated for 2.3 hours at 25 °C. D+6: the 6th leaf above D-leaf; D-6: the 6th leaf below D-leaf; W: basal white tissue; WG: basal white-green tissue; G: green tissue.

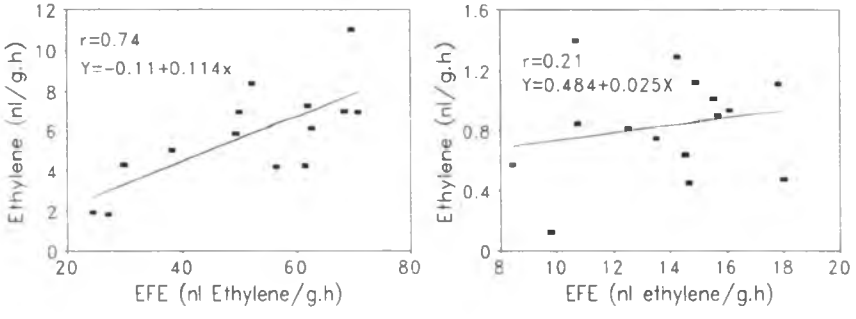

Figure 2. Relationship between EFE activity and ethylene production of D-leaf basal tissue (left) and stem apical tissue (right) of pineapple plants.

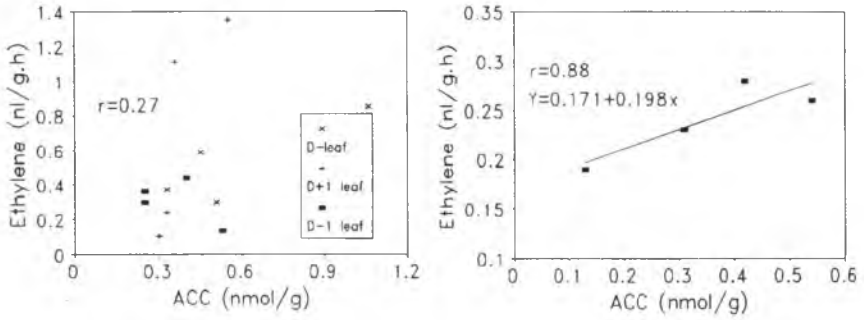

Figure 3. Relationship between ACC content and ethylene production of leaf basal tissue (a,left) and stem apical tissue (b,right) of pineapple plants.

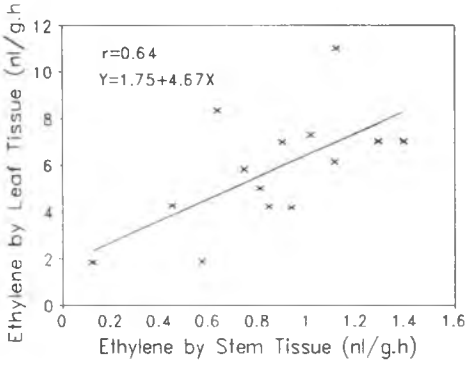

Figure 4. Relationship between ethylene production of leaf basal tissue and stem apical tissue of pineapple plants.

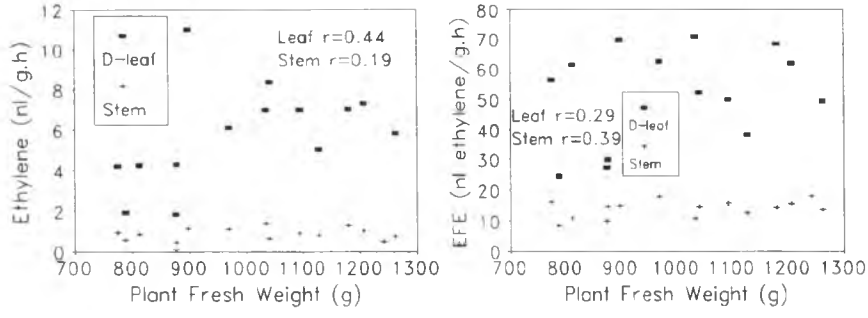

Figure 5. Ethylene production (a,left) and EFE activity (b,right) of plant tissue in relation to plant size.

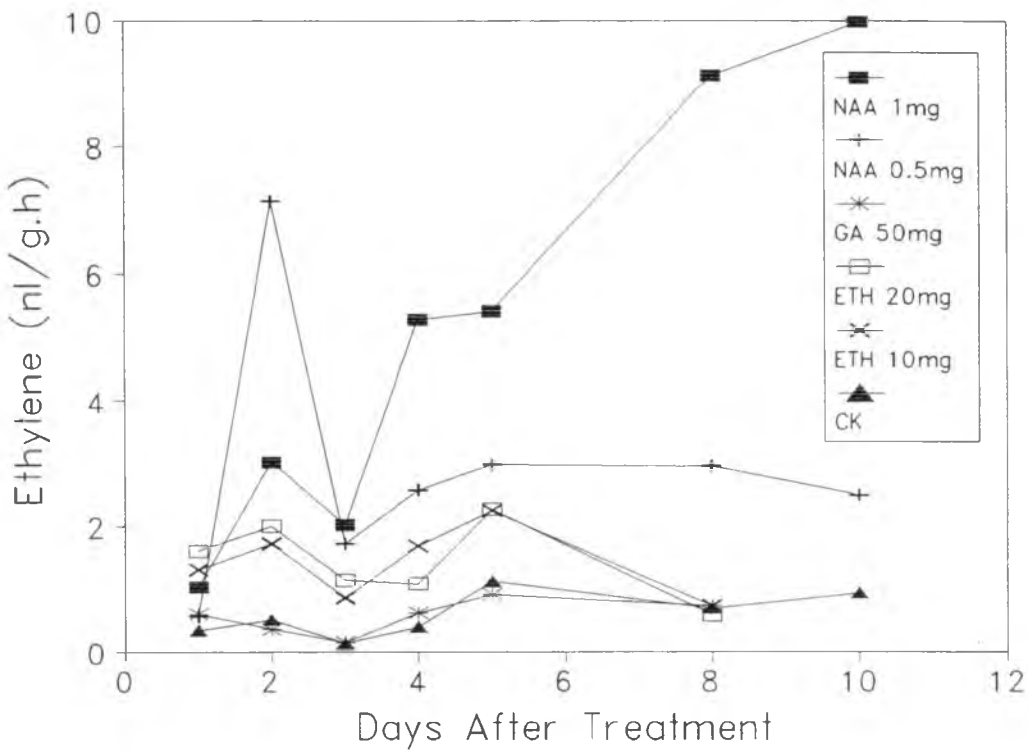

Figure 6. Effect of ethephon, naphthaleneacetic acid (NAA) and gibberellic acid (GA_3) on ethylene production by pineapple stem apical tissue.

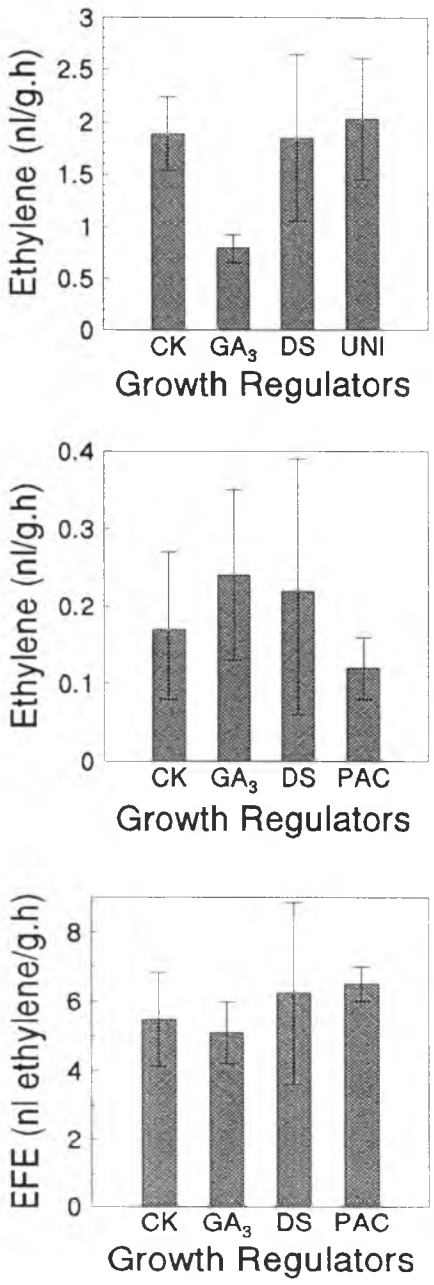

Figure 7. Effect of gibberellic acid (GA_3), dikegulac sodium (DS), uniconazole (UNI), and paclobutrazol (PAC) on ethylene production and EFE activity of stem apical tissue. The tissue was dipped (a,top) and incubated for 3 hours, or intact plants (b,middle and c,bottom) were treated and the excised tissue incubated for 2 hours.

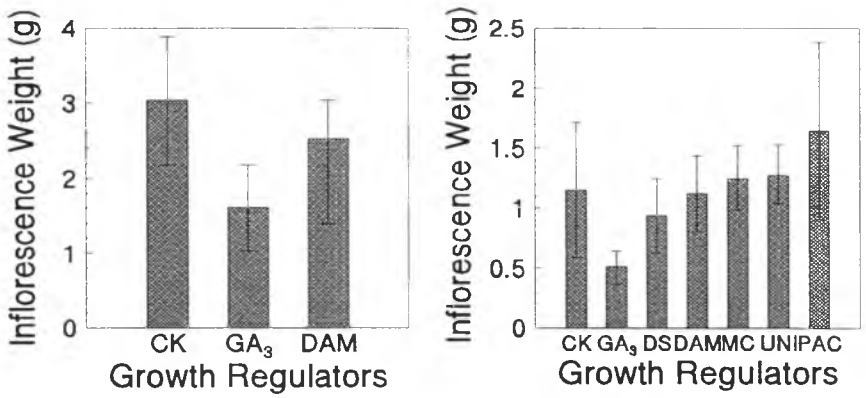

Figure 8. Effects of gibberellic acid (GA_3), daminozide (DAM), dikegulac sodium (DS), mepiquat chloride (MC), uniconazole (UNI), and paclobutrazol (PAC) applied prior to forcing on inflorescence weight of pot-grown plants (a,left) and field grown plants (b,right).

Ethylene production by stem tissue was significantly correlated with ethylene production by basal tissue ($r=0.64$) (Figure 4). Ethylene production by basal tissue was somewhat correlated with plant size ($r=0.44$) while that of the stem was not (Figure 5a); EFE activity in the leaf and stem were poorly correlated with plant size (Figure 5b).

3.2. *Effects of plant growth regulators on ethylene production and flowering*

The rate of ethylene produced by the plant tissue treated with ethephon was significantly higher than that produced by control plants and remained so for at least five days (Figure 6). However, without ^{13}C-labeled ethephon, it is not possible to distinguish between the ethylene produced by the plant and that released as a result of ethephon degradation. It is clear that the ethylene level in the tissue of ethephon treated plants was much higher than that in control plants. NAA stimulated ethylene

production by stem tissue even more than did ethephon and the effect lasted more than one week. GA_3 had no effect on stem tissue ethylene production. Plants treated with ethephon and NAA all initiated floral primordia; no primordia were produced by control plants or those treated with GA_3.

Ethylene production by stem tissue dipped in AOA was strongly inhibited. Treated tissue produced an average of 0.80 nl g^{-1} h^{-1} while tissue from controls produced more than 2.95 nl g^{-1} h^{-1} over the three h incubation period. Ethylene production by stem tissue dipped in a solution containing GA_3 was also was significantly inhibited (Figure 7a). However, when intact plants were treated with GA_3, ethylene production by excised stem tissue was not different from the control (Figure 7b). The growth retardants paclobutrazol, uniconazole, and dikegulac sodium also did not significantly affect ethylene production or EFE activity of stem tissue (Figure 7a,7b,7c).

Applying GA_3 or daminozide 8 days prior to ethephon forcing did not affect the susceptibility of greenhouse-grown plants to forcing, but the fresh weight of inflorescence in GA_3 treated plants was only 58% of the control (Figure 8a). The inflorescence weight of daminozide-treated plants was less than the control but the difference was not significant (Figure 8a).

When the growth regulators GA_3, dikegulac sodium, daminozide, mepiquat chloride, uniconazole, and paclobutrazol and STS, an inhibitor of ethylene action, were applied to field-grown plants to evaluate their potential to inhibit ethephon forcing of pineapple, only STS blocked ethephon forcing (86 % of the plants were not

forced). At the concentration used, STS caused some burning of young leaf tips. Other treatments had no effect on the susceptibility (sensitivity) of plants to ethephon forcing.

The inflorescence weight of GA_3 treated plants was less than controls while the growth retardants had no effect on inflorescence weight (Figure 8b). Peduncle length was markedly affected by the growth regulators with lengths in cm for controls, 27.4; GA_3, 58.5; paclobutrazol, 8.1; uniconazole, 11.0; dikegulac sodium, 21.0; mepiquat chloride, 26.6; and daminozide, 28.0 cm. Peduncles on plants treated with GA_3 were significantly longer than controls while paclobutrazol and uniconazole significantly shortened the peduncle. There was no significant difference in eye number per fruit, which was around 140, between treatments. At this time, there were no visible suckers or hapas in the control and GA_3-treated plants. For the other growth regulators, the number of suckers and hapas on 10 plants per treatment were: paclobutrazole, 19; uniconazole, 11; dikegulac sodium, 13; daminozide, 11; and mepiquat chloride, 2.

Six of the GA_3 treated plants bore fruit without a crown and other fruits had a strongly tapered transition zone between the bottom of the crown and the top of the fruit. Uniconazole treated plants produced multiple crowns. The effects of GA_3 and growth retardants on fruit growth, development, and quality need further investigation.

4. Discussion

Pineapple plants can produce ethylene naturally. The data suggest that ACC is a limiting factor in ethylene production by both stem and basal tissues because the rate of ethylene production from both tissues increased after dipping them in a solution containing ACC. The data also indicate that stem ethylene production is mainly controlled by ACC level while leaf basal tissue ethylene is also controlled by EFE activity. Although stem and basal tissue are different physiologically and biochemically, ethylene production by these two tissues are correlated. It is possible to use ethylene production by basal tissue as an indicator of ethylene production by stem tissue without sacrificing the plant.

There was no evidence of a relationship between the rate of ethylene production and susceptibility to forcing. Plants ranging in weight from approximately 0.8 to 2.0 kg had approximately the same rate of ethylene production and were equally susceptible to ethephon forcing. Other data (K. Liu and D. Bartholomew, unpublished data) showed that plants weighing less than about 0.4 kg produced less ethylene than plants weighing 0.8 kg or more; the small plants could not be forced while the larger ones were readily forced.

The effect of AOA on ethylene production by intact plants needs to be expanded to the exploration of its effects on floral initiation; especially if natural flowering can be induced in a predictable manner. One of the major impediments to progress in studying the physiology of flowering of pineapple is our inability to consistently produce plants that are not susceptible to ethephon forcing. There may

be some benefit in further study of the effect of growth retardants on flowering. These chemicals can increase plant resistance to stress conditions (Davis and Curry, 1991), and some growth retardants such as uniconazole (Kraus et al. 1991), dikegulac sodium (De Greef et al. 1989), and daminozide (Gussman et al., 1993) have been reported to reduce ethylene production or the conversion of ACC to ethylene in other plants.

All evidence indicates that ethylene is directly involved in floral initiation because 1) the pineapple plant can produce ethylene; 2) ethephon and NAA increased stem tissue ethylene level, and induced plants to flower; 3) STS has been shown to block ethylene action (Abeles, et al., 1992) and plants treated with STS prior to forcing remained vegetative. The relationship between natural flowering, plant susceptibility and ethylene production needs further investigation.

Sanford and Bartholomew (1981) reported that application of silver nitrate a few hours prior to ethephon forcing decreased flowering percentage. Millar-watt (1981) also found that silver nitrate significantly reduced natural flowering. Silver ion has been reported to effectively block ethylene action (Abeles, et al., 1992) and may inhibit natural flowering in pineapple. In order to successfully inhibit precocious flowering of pineapple, the optimal concentration and time of application all need to be studied.

The fact that GA_3 reduced inflorescence size (while increased peduncle length), may indicate it involved in floral development. It isn't clear whether the smaller inflorescence was the result of a direct retardation of floral growth or a reallocation of

assimilates to other plant parts. The internal factors controlling plant sensitivity to forcing agents and the relationship between environmental factors and plant susceptibility remain to be explored.

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